The Assessment Level of Fluoride Intake/Exposure using “3-Day Dietary Diary” & “2-Day Duplicate” methods

N. Omid

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Declaration

I hereby declare that the work presented in this thesis is entirely my own and that, to the best of my knowledge, has never been published or presented for the award of any other degree or diploma of the university or other institute of higher education.

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Abstract

Background: Studies of assessing dietary fluoride intake in children have employed different dietary methods mainly "2-day duplicate" and "3-day food diary" methods. However, none of these methods have been validated or standardised.

Main aims: The main aims of the current study were to develop a better understanding of strengths and weaknesses of dietary assessment methods "2-day duplicate plate" and "3-day food diary" by comparing dietary fluoride intake estimated by each method and evaluate the validity of the two methods for estimating dietary fluoride intake in young children.

Methods: Sixty one healthy 4-6 year old children living in fluoridated area of the north-east of England since birth were recruited via 10 primary schools. Dietary information was collected using "2-day duplicate plate" and "3-day food diary" methods. Two 24-h urine samples and two samples of post brushing expectorate (a mixture of saliva, toothpaste and water used to rinse after brushing) from each child. Completeness of 24-h urine samples was checked using urinary excretion of creatinine and urinary flow rate. Validity of the two dietary assessment methods was checked by measuring urinary excretion of nitrogen and potassium as independent validity checks. Total daily fluoride intake from diet and toothpaste ingestion and urinary fluoride excretion was determined for each child.

Results: All participated children completed all aspects of the study. According to the validity criteria, dietary data of 58 (95%) children, when collected by the 3-day food diary, were considered valid. However, when the dietary data were collected by the 2-day duplicate plate method, the data for 46 (75%) children were viewed as valid. Mean total dietary fluoride intake was 0.533 mg/d by the 3-day food diary method and 0.583 mg/d by the 2-day duplicate plate method. No statistically significant difference in total dietary fluoride intake was observed between the two methods.

The mean difference in estimated dietary fluoride intake by the two dietary assessment methods was -0.050 mg/d with 95% limits of agreement of -0.501 to + 0.401 mg/d.

Conclusion: Either the 3-day food diary or the 2-day duplicate plate method can be used when investigating mean total daily dietary fluoride intake of a population. However the methods cannot be used interchangeably at the individual level.

Chapter 1 Introduction

1.1. Fluoride and environment

Fluoride and fluorine are two generic terms which are frequently used interchangeably in the literature. Fluoride can be found naturally in both water and air. While it represents 0.06 to 0.09% of earth's crust, water contains fluoride in various concentration (WHO 1994).

Although the concentration of fluoride in seawaters is significant at levels of 0.8-1.4 mg/l, most lakes, rivers and artesian wells contain a fluoride concentration of less than 0.5 mg/l. Concentrations as high as 95 mg/l have been identified in the United Republic of Tanzania (WHO 1994). Waters at the foot of high mountains in areas with geological deposits of marine origin are usually found to contain higher fluoride concentrations. The highest natural fluoride concentration ever found and recorded was at 2800 mg/l in Lake Nakuru in the Rift Valley in Kenya (WHO 1994).

Fluoride is emitted into air in the form of particulate or gaseous. The natural sources of fluoride in air are volcanic dusts and gases emitted from volcanic activity as well as dusts from fluoride containing soils. Human activities such as domestic burning of coal fire and gaseous industrial waste can influence airborne fluoride (WHO 1994). Most of fluoride in atmosphere is in the form of hydrogen fluoride (HF) which is absorbed rapidly into the lungs. However, exposure from atmosphere is negligible except in heavily polluted areas (Hodge & Smith 1977).

Fluoride concentration of foods varies with the unprocessed food reported to have a range of 0.1 to 2.5 mg/kg (WHO 1994). However, the majority of foods have fluoride concentration of less than 0.5 µg/g (Taves 1983). The exceptions to this are tea and fish. Tea leaves are rich in fluoride which is readily released into the water upon brewing (Whitford 1994, Fejerskov *et al.*, 1996). The fluoride concentration of prepared/processed meals depends on not only the concentration of fluoride in the soil but additionally its concentration in water used for their processing. However, the inclusion of bones while processing food may contribute to higher level of fluoride in the food (WHO 1994). Therefore processed and mechanically deboned meat and poultry products which could contain quantities of ground bone, may contain 0.6 to 10.6 mg/kg fluoride (Wiatrowski *et al.*, 1975, Singer & Ophaug 1979).

Fluoride concentration of beverages which is dependent on the fluoride concentration of water used for their preparation, ranges from 0.1 to 1.4 µg/g except for tea.

1.2. Fluoride metabolism

1.2.1. Fluoride ingestion and absorption

The sources of fluoride ingestion includes both dietary sources such as water, beverages, food and fluoride supplements and non-dietary sources such as toothpastes, mouth rinses, gels and drops.

Fluoride absorption starts in the oral cavity at a substantially low rate and accounts for only 1% of total fluoride absorption. In the absence of some dietary components such as Calcium (Ca) and Magnesium (Mg) almost 80-90% of ingested fluoride is absorbed from gastrointestinal tract. Absorption from stomach accounts for 20-25% of ingested fluoride (Whitford 1996). Absorption from both oral cavity and stomach is pH dependent. Low pH favours fluoride absorption while alkalinity decreases fluoride absorption. The remaining fluoride that enters small intestine is absorbed rapidly as fluoride ion by non-pH dependent diffusion (Whitford & Pashley 1984).

The half time for absorption is approximately 30 minutes and peak plasma concentration occurs within 30-60 minutes. Once the majority of ingested fluoride is absorbed the rate of its clearance from plasma exceeds the rate of absorption.

Fluoride absorption is influenced by a number of factors. Dietary components such as Ca, Mg, and Aluminium (Al) could combine with fluoride and form insoluble salts which reduces fluoride absorption from the gastrointestinal tract. In contrast if fluoride is ingested in a readily soluble form such as sodium fluoride, absorption is almost complete. Therefore, fluoride absorption varies with the physical form of its administration, the solubility of the fluoride salt and presence or absence of large amounts of calcium in gastrointestinal tract .

1.2.2. Fluoride distribution

The distribution of fluoride is through plasma. The inorganic (ionic) form of fluoride is not bound to proteins or any other components in plasma or cell membranes or sub-cellular structures. It is detectable by ion-selective electrode and is of biological interest. It presents in intracellular fluids of soft tissues and its

concentration is in a steady-state relationship with the concentration in plasma. The non-ionic component consists of several fat-soluble organic fluorocomponenets, most or all of which bind to plasma proteins and are not detectable by the fluoride electrode. The biological significance of this component has not been determined. Ionic fluoride concentration of plasma is proportional to fluoride intake and so its measurement is a reasonably reliable indicator of fluoride exposure (Whitford 1996). Fluoride is rapidly removed from plasma by mineralised tissue where it is exchanged with anions such as hydroxyl ion, citrate and carbonate. Fluoride is deposited in calcified tissues as 99% of total body fluoride can be found in bones and teeth (Murray & Rugg-Gunn 1982, WHO 1984, Murray 1986a).

The rate at which fluoride is removed from plasma depends on the stage of skeletal development which is greater in developing skeleton than in mature bones. This is largely due to greater surface area provided by loosely organised bone crystallites which increases fluoride clearance from plasma compared to mature bones. This range varies from 60% (Ekstrand & Whitford 1988, Whitford 1990) to 80% (Hargreaves *et al.,* 1970, Ekstrand *et al.,* 1994).

In summary, the degree of fluoride uptake by skeleton and dentition depends on the amount of ingested and absorbed fluoride, duration of exposure, type, location and metabolic activity of the tissue as well as age of the individuals (Murray 1986a).

1.2.3. Fluoride excretion

More than 90% of ingested fluoride is usually absorbed and the remaining 10% is excreted through faeces (Ekstrand *et al.*, 1984, Ekstrand 1994). The major route of fluoride removal from the body are the kidneys. About 60% of absorbed fluoride each day is excreted in the urine in adults and that for children is 45% (Villa *et al.*, 2010).

Renal excretion of fluoride varies among the individuals and is affected by alterations in glomerular filtration rate, urinary flow rate and urinary pH (Ekstrand *et al.,* 1982, Spak *et al.,* 1985).

Renal tubular reabsorption of fluoride is also pH dependent and occurs by diffusion of hydrogen fluoride (HF) (Whitford *et al.,* 1976). With the acidic condition of tubular fluid ionic fluoride is converted to HF which is diffused across the tubular epithelium. While the pH of the tubular fluid is high, the

proportion of HF is lower than the proportion of ionic fluoride. Thus the ionic fluoride remains within the tubule and excreted (Whitford 1990). Therefore, any factors that alter urinary pH could have direct effect on tissue fluoride concentration. One of these factors include diet composition such as meat based or vegetarian based diets which are associated with higher and lower fluoride retention, respectively (Ekstrand *et al.,* 1982). Other factors are certain drugs, respiratory or metabolic disorders, level of physical activity and high altitude (Whitford 1990).

Urine pH and flow rate are also related since the increase in urinary flow would dilute the urine and concurrently increases its pH.

Physiological changes associated with residence at high altitude which might be due to hypoxia in high altitude areas, could reduce the urine pH and consequently the renal excretion of fluoride which in turn increases the body fluoride concentration.

1.3. Fluoride and oral health

The early investigation by Trendly Dean (Dean *et al.,* 1950) demonstrated that the level of 1 mg/l of fluoride in drinking water was optimum to reduce dental caries while minimising dental fluorosis. Ever since fluoridation of water supply has become one of the most important public health measures. The encouraging results coming from this measure promoted incorporating fluoride in other products and as a result fluoridated toothpastes, mouth rinses and tablets became available.

Fluoride's mode of action to reduce dental caries is both systemic and topical. However, the main mechanism of fluoride action relies on its topical use which is by: i) inhibiting demineralisation by which if fluoride presents in plaque fluid when acids are produced by bacteria, fluoride will penetrate along with the acids at the subsurface, adsorb to the crystal surface and protect crystal surface from dissolution (Featherstone 1999) and ii) promoting remineralisation. Remineralisation naturally occurs since saliva is supersaturated with dental minerals. Presence of fluoride in solution during dissolution of hudroxyapatite makes the solution highly supersaturated with respect to minerals and speed up remineralisation.

Fluoride's mechanism of action to control caries could also be systemic such as fluoridated water. However, fluoride in water despite being regarded to have

systemic effects, is primarily through topical effect due to the direct contact of fluoride with the tooth surface prior to ingestion while it is in oral cavity or redistributed to the oral cavity by saliva. This topical action has been attributed to higher frequency of fluoride contact with the tooth structure which is provided by higher consumption of fluoridated water (Wasdinb & Adairb 2002). The systemic effect of fluoride to prevent caries occurs during developing enamel by pre-eruption fluoride uptake in the crystalline structure, its adsorption on the crystal surface or its presence in the enamel fluid (Singh *et al.*, 2003, Singh *et al.*, 2007).

1.4. Fluoride and dental fluorosis

Excessive fluoride ingestion during enamel development has adverse effect and results in developing dental fluorosis. Dental fluorosis is a dose response effect and depends on duration of exposure as well as timing of exposure. The most susceptible time for developing dental fluorosis is during the transition or early maturation stages of the development of enamel (Burt 1992). The results are white/ brown spotty staining of enamel. Studies by Dean (Dean 1956) showed that the level of 1 ppm caused very mild fluorosis with the prevalence of 10 to 12%, while at the level of 2 ppm the prevalence of the fluorosis increased to 50% with 5% at moderate fluorosis (Clarkson & McLoughlin 2000). Chronic exposure to high doses of fluoride could result in skeletal fluorosis. It is associated with an increase in bone mass and involves all bones. Studies of fluoride balance suggested that endemic skeletal fluorosis occurs in individuals (adults) with a daily intake of more than 8 mg (WHO 1984).

1.5. Fluoride balance

Knowledge on the availability of fluoride both during enamel development and post-eruptive period facilitates the assessment of potential developing of dental fluorosis and protection from caries respectively (WHO 1994).

Nowadays, the availability of multiple sources of fluoride has raised the concern that fluoride exposure might have exceeded the optimum level. Considerable research has therefore been given to assessing optimum fluoride intake. In a review by Burt (Burt 1992) the uppermost limit of total fluoride intake from “all sources” including dietary (food and beverages), non-dietary (toothpastes, mouthrinses and gels) and fluoride supplements was estimated to be 0.05-0.07 mg per kg body weight per day (mg/kg bw/d). However, it has been argued that the

threshold level of fluoride intake for the development of dental fluorosis may be as low as 0.03-0.04 mg/kg bw/day (Baelum *et al.,* 1987, Fejerskov *et al.,* 1987).

1.6. **Scope of the thesis**

There is a narrow margin between the optimal fluoride intake to prevent dental caries and excessive intake to cause dental fluorosis. As a result, monitoring fluoride exposure is an important part of community-based fluoridation programme. The main sources of fluoride exposure are diet and toothpaste ingestion. Diet could contribute up to 70% of total daily fluoride intake of children up to age 6 (Levy *et al.,* 2003). Therefore, many studies have focused on assessing dietary fluoride intake using different dietary assessment methods. However, there is a lack of information on validation of dietary assessment methods in all studies.

1.7. Thesis outline

This thesis is divided into 11 chapters. Chapter one describes some basic principles to the study and proceeds to Chapter 2 in which the literature is reviewed followed by identification of the research aims and objectives in Chapter 3. A pilot study was conducted to find an accurate and quick method for analysing expectorated saliva, toothpaste and rinse. This study is presented in Chapter 4. Chapters 5, 6 and 7 present the methods of recruitment, data collection, general results and validation of dietary assessment methods. Chapter 8 describes and discusses fluoride intake data collected by the two dietary assessment methods: "2-day duplicate" and "3-day food diary". In Chapter 9 urinary excretion of fluoride from two collections are presented and discussed. The fluoride intake and excretion data are then compared between the two methods/collections at the group and individual levels in Chapter 10. An overall discussion from the findings of this study followed by conclusion and suggestions for future research are presented in Chapter 11.

Chapter 2 Literature review

2.1. Introduction

A critical review of available literature is presented in this chapter with a view to identify gaps in the knowledge. This review explores literature in areas of dietary assessment methods, their advantages and limitations with a focus on the methods used for the assessment of fluoride intake. Further, this chapter examines literature on validation of dietary assessment methods, fluoride intake and excretion studies conducted in children.

2.2. Dietary assessment methods

Generally, methods of assessing the nutrient intakes of individuals can be divided into two categories: "Prospective" in which food is recorded as it is eaten and "Retrospective" which involves the recall of foods eaten previously.

2.2.1. Prospective methods

The main dietary assessment methods which investigate dietary intake of individuals prospectively are "food records" and "duplicate" methods

- **Food Record**

In this method the actual intake of food and beverages consumed during specific time period is recorded by the respondent. The time period is usually 3, 5 or 7 consecutive days (Thompson & Byers 1994). However, recording for more than 4 consecutive days may result in decrease in reported intakes (Gersovitz *et al.*, 1978). In this method, details of consumed food and drinks including brand name, cooking and preparation methods, ingredients of mixed dishes as well as time of consumption is recorded. Therefore the method allows the sources of food to be identified. In addition, portion sizes of consumed food and drinks are weighed either by a scale or household measures (such as cups, tablespoons), or estimated using models or pictures. The records are administered at the time of consumption and are less likely to be missed out. Besides, the method doesn't alter dietary habits. A 7-day food record has been suggested as the "gold" method for validating other methods (Willett 1998). However, a decrease in validity of the collected information has been reported when the number of days increased.

Therefore, the use of 7-day food records for validation purpose is limited due to a significant increase in incomplete records (Gersovitz *et al.,* 1978).

Weighed food records have traditionally been used for the assessment of dietary intakes in the UK in national surveys such as the National Diet and Nutrition Survey (NDNS) (Gregory *et al.,* 2000). The NDNS survey provided a comprehensive, cross-sectional picture of the dietary habits of 1701 children aged 4-18 years using a 7-day weighed dietary record method.

A 3-day weighed food record was also employed in a longitudinal study in Germany (DONALD) to assess the protein intake of 439 children and adolescents (Bokhof *et al.,* 2010).

However, food record has some limitations. It may present a burden to the participant and researcher alike. Respondent should record detailed description of food while the researcher should check the records for completeness and missing entries with the participants and use two or three dimensional food models to aid participants to quantify food portions (Brunner *et al.,* 2007). Besides, it requires respondents to be literate, motivated and fully co-operative. Therefore, it can potentially limit the use of the method in some populations such as those with low literacy rate which makes it subject to bias both in the selection of the sample and measurement of the diet (Thompson & Subar 2001a). Other sources of errors have been addressed as coding errors and incorrect recording of consumed food (Anderson 1995).

- **Duplicate diet**

This method involves retaining an identical portion of all foods and drinks throughout the day by respondents. The identical portions are then weighed and analysed by the researchers.

Several studies both in adults (Bingham *et al.,* 1982, Kim *et al.,* 1984, Bro *et al.,* 1990) and children (Goshima *et al.,* 2008, Sugiyama *et al.,* 2009) have employed this method to assess nutrient intakes. This method has been regarded as one of the most accurate ways of sampling the diet since the diet is duplicated at the point of eating (Basiotis *et al.,* 1987, Guha-Chowdhury *et al.,* 1996). In addition the actual foods/ drinks consumed by participants are analysed directly without the use of food consumption tables. It is therefore an objective measure of dietary assessment which is not subject to the limitations of food composition tables,

inaccurate recording of the amounts, inadequate coding and non-inclusion of a given food type in the food consumption tables.

However, the major disadvantage of this method is the cost of duplicating which makes it unsuitable for large-scale studies. Since this method poses a huge burden on participants, there is a greater possibility of altering dietary habits to ease the burden. This method requires participant to provide a complete duplicate of consumption. Therefore, a high degree of co-operation and motivation is required from participants. Another disadvantage of the method is that the sources of nutrient intake cannot be identified since food and drinks are pooled.

2.2.2. Retrospective methods

These methods collect dietary data over a period of time such as a 24-h, week or year. They are mainly designed to gain information in large epidemiologic studies due to low cost involved in dietary data collection. However, as they rely on memory, information is more likely to be omitted which make these methods unsuitable for particular populations such as children and elderly who have difficulties with memory (Biro *et al.*, 2002). These methods are easy to administer and pose minimum burden for participants.

The methods are as follow:

- **24-h Recall**

This method involves a short interview in that a trained nutritionist or other professional asks participants to list everything participants consumed during the previous day. It can also be conducted via paper records, telephone interview or computer-assisted program. The main advantage of 24-h recall is that it generally has a higher response rate and provides a detailed description of the food including; brand names, composition and preparation of the foods as well as the portion size. Thus the complete nutrient intake can be calculated for the designated day (McPherson *et al.*, 2000). However, this method requires a trained dietician to interview subjects, assess portion weights and make appropriate enquiries about type of food, drinks consumed as well as those which might be omitted such as snacks.

A single 24-hour recall is not appropriate for estimating the habitual intake of an individual due to day-to-day, intra-individual variation in food intake. Therefore, multiple records should be collected to accurately estimate usual nutrient intake

(Rockett & Colditz 1997, McPherson *et al.,* 2000). Repeated 24-h recall was conducted to study day-to- day variations within individuals over a 12 month period (Balogh *et al.,* 1971). In their study Balogh et al. found that day-to-day variation within individuals was very high and in fact a very large number of 24-hour recalls over an extended period of time was necessary to represent adequately the average or usual value for an individual. In studies with children the use of repeated 24-h recall puts a huge burden on parents or care givers since they need to provide this information over a period of time.

This method might be subject to bias in recalling the food and estimating portion sizes. However, it is quick, simple and inexpensive which makes it more suitable for clinical dietetic studies (McPherson *et al.,* 2000).

- **Market basket collection**

"Market basket" collection is an approach by which the representative diet of the study group is collected to estimate the dietary intake of certain nutrients. The food collection comprises of several composite food groups found in the diet, according to shopping guidelines. The guidelines are usually derived from a household consumption survey reflecting the actual 14-28 days consumption of various food items by the study group. The amount of the target nutrient is then determined in each composite by relevant analytical methods.

This method has been widely employed to investigate the level of some nutrients or even contaminants such as pesticides intake from food in large epidemiological studies (Schoof *et al.,* 1999, Kiviranta *et al.,* 2004, Schecter *et al.,* 2006, Darnerud *et al.,* 2006). Since a survey on household food consumption should be conducted for at least 14 days prior to analysis, the method poses a huge burden on investigators. In addition, this method could not be accurate as a difference between adults and children in the type and the amount of consumed food as well as household food waste is not taken into consideration when estimating the intakes.

- **Food Frequency Questionnaires (FFQs)**

The food frequency questionnaires measure usual food intake of a group of individuals over a specific period of time usually 6 months to one year. Specific food items are listed with a selection of options for reporting how often each food is consumed. The food list may contain only a few items or up to 200 items. In addition to estimate the quantities of food or nutrient intakes, some FFQs ask

respondents to provide information on portion size (semi quantitative FFQ). To do this, respondents can refer to a picture atlas of food portions or standard portion size described on the questionnaire (Nelson *et al.*, 2007). Nutrient intakes are then determined by multiplying food frequency scores for individual items by the nutrient content of the local standard portion or estimated portion size (Bingham *et al.*, 1988).

This method has been used in many epidemiological studies involves large number of adult participant to investigate the relationships between dietary intake and diseases outcome (Willett *et al.*, 1985, Subar *et al.*, 1994, Rothenberg 1994, Haraldsdottir *et al.*, 1994, Bingham *et al.*, 1994, Tsubono *et al.*, 1995, Porrini *et al.*, 1995, Pisani *et al.*, 1997). Several studies have also used the methods in children (Byers *et al.*, 1993, Blum *et al.*, 1999, Marshall *et al.*, 2003).

There are some elements in designing the FFQs of which if not considered carefully could offer disadvantage to the method. These elements are the length of the questionnaires, type of questions such as closed versus open-ended response, portion size inclusion, seasonality, and time frame that the respondents should recall the food and drinks they consumed. Some questionnaires list more than 100 individual items with some questions about portion sizes. Although the quantity of food consumed is very important in determining the dietary intakes for FFQ, some prefer to use FFQ without the additional burden of reporting portion size (Willett 1998) since the frequency is a greater contributor to the variance in intake of most foods rather than serving size (Heady 1961). However, portion size and frequency can be incorporated into one question in some FFQs. Inclusion of such information in the questionnaire generally depends on the study objectives and population characteristics (Willett 1998).

One of the advantages of the FFQs is that they are primarily aimed to provide a practical and cost-effective way of long term dietary data collection from large numbers of respondents. Thus, they are designed to be self- administered with 30-60 minutes to complete (Rutishauser 2007).

The major disadvantages of FFQ are: i) it contains a significant amount of measurement error, and ii) little details are collected on the characteristics of consumed food and drinks such as preparation.

- **Diet history**

The diet history quantitatively measures individual's habitual dietary intake over a specified time period by a) determination of the actual food intake of the preceding day by an interview and b) cross checking of this information by food groups. The interview usually takes one hour and requires skilled staff. The major strength of this method is its ability to assess meal patterns and details of food intake rather than intakes for a short period (Thompson & Subar 2001b).

Besides, the method does not alter dietary habits and doesn't require the respondent to be literate.

However, the disadvantage of this method is that the recall may not be precise and it requires a highly trained interviewer. Several studies have used this method in adults (Van Staveren *et al.,* 1985, Visser *et al.,* 1995, Black *et al.,* 2000). However, with children the information obtained by this method relies on parents since the ability of children to co-operate is limited. In a study on a group of 3-18 year olds using this method to compare energy intake derived from 7-day diet record and diet history showed that diet history was biased towards overestimation in most age groups with the lack of precision at individual levels. However, the method was found to be more representative of habitual intake compared with diet record (Livingstone *et al.,* 1992).

2.2.3. Methods used for the assessment of fluoride intake

Dietary fluoride intake of children has been investigated using different dietary assessment methods:

'Market basket' collection method was used to assess dietary fluoride intake of children in the USA (Ophaug *et al.,* 1980, Ophaug *et al.,* 1985): A 2-week supply of food for an average 6 months old infant and 2 year old toddler was estimated from the survey conducted by the United States Department of Agriculture, Food and Drug Administration (United States Department of Agriculture 1968). Daily fluoride intake of infants and toddlers was then calculated based on the estimates of daily food consumption and the fluoride content of each composite food (Ophaug *et al.,* 1980).

The fluoride intake of 4 year old Hungarian children was assessed using a 7-day food record (Schamschula *et al.,* 1988). Dietary fluoride intake of infants and toddlers in the US was estimated using a 3-day food and drink diary (Pang 1992,

Levy 1995). A 3-day food diary with a face-to-face interview on the 4th day was used to assess dietary fluoride intake of children in Iran (Zohouri & Rugg-Gunn 2000b) and England (Maguire *et al.,* 2007).

Duplicate plate method has been widely used to measure fluoride intake in children (Guha-Chowdhury *et al.,* 1996, Murakami *et al.*, 2002, Franco *et al.*, 2005, Nohno *et al.,* 2006).

The FFQ was used in the recent longitudinal Iowa study to investigate dietary fluoride intake of children in Iowa (Levy *et al.,* 1998, Levy *et al.,* 2001, Levy *et al.,* 2003). A semi-quantitative FFQ was also used in Brazil to estimate dietary fluoride intake of 2-6 year old children (Miziara *et al.,* 2009).

2.3. Validation of completeness of 24-h urine collections

Due to difficulties in obtaining accurate dietary information by dietary assessment methods, use of a biochemical measure of exposure to a nutrient of interest has been suggested as an alternative method of assessment (Day *et al*., 2001). Biomarkers reflecting nutrient intakes can be found in various biological media, including urine, faeces, blood, hair, and nails. Since the majority of biomarkers can be found in urine, it is the most frequently employed biological medium in nutritional epidemiological studies. In addition, collection of urine is relatively convenient and non-invasive. However, the completeness of 24-h urine should be verified in order to avoid drawing incorrect results and conclusions.

Different markers have been suggested for checking the completeness of 24-h urine. These markers can be categorised into two major groups;

1) Externally induced markers

2) Internally produced markers

2.3.1. External markers

External markers involve oral application of certain substances, followed by measuring the amount of substance excreted through urine. The two different substances used are lithium and para-amino benzoic acid (PABA).

- Lithium

Lithium has been known as a suitable external biomarker due to its very low concentration in the diet (Sanchez-Castillo *et al.*, 1987). Since, it is completely excreted in the urine the 24-h urinary excretion of lithium reflects its daily dose.

In order to check the completeness of 24-h urine, lithium tagged salt is given to participants a few days before the urine collection day (Von Houwelingen *et al.*, 1987). Urine sample with >95% recovery of lithium are then classified as complete (Bingham 2003). However, the use of lithium has the disadvantage that with increased fluid intake the recovery of lithium from urine will be decreased which may lead to false conclusion that the participant might have provided incomplete urine sample (Amdisen 1977).

- PABA

In order to check completeness of 24-h urine collections using PABA, it should be given to participants with the morning, mid-day and evening meal. PABA is actively absorbed and excreted quantitatively within 24 hours. Therefore, 24-h collections with <85% of the ingested PABA will be regarded as incomplete, which can be due to either not taking the tablet or missing one or more specimens from the collection (Bingham *et al.,* 1992). PABA has been used in methodological studies carried out in the UK both in adults and children (Bingham *et al.,* 1997, Black *et al.,* 1997, Bates *et al.,* 2010). However, information about the use of both markers in children is limited. This might be due to the concerns among parents regarding the side effects and safety of these substances. Besides, some children may refuse to take any tablets.

2.3.2. Internal markers

Internal markers do not require any tablets to be taken at certain times, or the timing of doses to be recorded. They place fewer burdens on participants and therefore are more acceptable for children.

- Creatinine

Creatinine is produced from creatine which is mainly found in muscle tissues. Although creatine in the diet mostly comes from meat, more than half of the body creatine is synthesized in the liver and kidneys from the amino acids arginine, glycine and methionine. In healthy normal individuals, about 40% of muscle creatine exists as free creatine while the rest is found in the form of creatine phosphate (CP), which provides a constant supply of adenosine triphosphate (ATP) as a source of energy for muscle contraction. Creatinine is spontaneously produced from creatine during the dephosphorylation of creatine by an irreversible non-enzymatic reaction. It has no specific biological function and is

steadily released from the muscle cells and excreted via the kidneys with minimum re-absorption (Litchford 2008).

Total 24-h urine creatinine has been suggested as a reliable measure of completeness of the 24-h urine collections for healthy individuals with no muscle tissue loss due to dietary restrictions or injury. Although the ranges of 24-h urinary creatinine excretion has been well established in adults, there are few reference values of 24-h urinary creatinine excretion in children and adolescents (Tietz 1995, Marthaler 1999, Remer *et al.,* 2002, Avner *et al.,* 2004). Since the major determinant of urinary creatinine excretion is muscle mass, the reported reference values are based on weights and/or heights. Available creatinine reference values have been summarised in Table 2.1.

Table 2.1 Summary of proposed creatinine values based on different criteria

Author	Proposed formula or range	Parameters used
Viteri & Alverado (1970)	$CE\ (mg/24h) = 0.0817H^2(cm) - 8.01H + 252$	Height
Tietz (1995)	8-22 mg/kg bw/d	Weight, age
Harms & Scharf (1997)	$CE\ (mg/kg\ bw/ = 15 + [0.5 \times age(yrs)] \pm 3$	Weight age
WHO (Marthaler 1999)	0.1-1.5 mg/ml	All ages
Remer (2002)	Male - 4-5yrs 17.08mg/kg bw/d - 6-8yrs 19.45mg//kg bw/d Female - 4-5yrs 16.06mg/kg bw/d - 6-8yrs 18.09mg/kg bw/d	Weight, age, sex
Avner (2004)	<2yrs 7.1-9.9 mg/kg bw/d 2-8yrs 12.2-21.2 mg/kg bw/d 9-18yrs 14.9-23.9 mg/kg bw/d	Weight, age

In 1970, Viteri and Alverado (Viteri & Alvarado 1970) suggested an empirical formula for expected urinary creatinine excretion: "$CE = 0.0817H^2 - 8.01H + 252$"; where CE is the urinary creatinine excretion (mg/24h) and H is the height (cm). The formula was based on reported normal height data by Stuart and Stevenson (Stuart & Stevenson 1959) and reported creatinine excretion rates by Daniels and Hejinian (Daniels & Hejinian 1929) which are quite old and out of

date references. In addition, in their formula, the between and within-individual variation in creatinine excretion has not been considered. For example, creatinine excretion can be affected by the variation in diet and since most creatine in the diet comes from meat, variation in meat consumption might affect creatinine excretion.

Tietz (Tietz 1995) suggested a wide range of creatinine excretion from 8 to 22 mg/kg bw/d to cover between and within-individual variation in meat consumption. This reference is based on body weight of which changes in muscle mass during growth were taken into account.

In the proposed formula by Harms and Scharf (1997) (Table 2.1) although age was included, the range of ±3 was not wide enough to cover the between and within-individual variation in meat consumption.

A creatinine reference range of 0.1-1.5 mg/ml has been proposed by the WHO (1999). However, in this reference none of the parameters of age, weight and height of individuals which are the major determinant of creatinine excretion have been considered.

In an extensive study, Remer and colleagues (Remer *et al.*, 2002) developed anthropometry- based age- and gender- specific creatinine reference values for a large population of healthy white children in developed countries. They established five narrower age groups of 3, 4-5, 6-8, 9-13 and 14-18 years in order to control the influence of age on the ratio of creatinine to body weight. The ranges (which are 5^{th} and 95^{th} percentiles) are gender specific reference values for body weight and related 24-h urinary creatinine excretion during growth (Table 2.2).

Table 2.2 Mean (range) creatinine excretion references values proposed by Remer 2002

Age	**Mean (range) creatinine excretion [mg/kg bw/d]**	
	Boys	Girls
3 yrs	15.16 (10.86-21.38)	14.36 (8.93-20.58)
4-5 yrs	17.08 (11.99-24.20)	16.06 (12.33-21.15)
6-8 yrs	19.45 (15.16-25.56)	18.10 (12.44-26.58)
9-13 yrs	20.58 (11.31-27.71)	19.34 (13.23-27.60)
14-18 yrs	22.73 (13.23-33.25)	20.58 (14.59-26.92)

The authors also observed that minimum creatinine excretion for 95% of children older than 3 years was 11.3 mg/kg bw/d. Therefore, a cut-off point of 11.3 mg/kg bw/d was suggested as an acceptable creatinine excretion rate.

They also suggested that the exclusion should not be based on one criterion only, and a second criterion should be considered due to limitations of each criterion.

Avner and co-workers (Avner *et al.,* 2004) suggested a range for creatinine excretion from 7 to 24 mg/kg bw/d for individuals younger than 19 years old. They divided the age range into three subgroups of <2, 2-8, and 9-18 years with a reference range for each age group (Table 2.3).

Table 2.3 Creatinine excretion reference values suggested by Avner

Age (yrs)	μmol/kg bw/d	mg/kg bw/d
<2	62-88	7.1-9.9
2-8	108-188	12.2-21.2
9-18	132-212	14.9-23.9

- Urine flow rate

Urine flow rate is another commonly used marker to validate the completeness of 24-h urines. Flow rates of 5-160 ml/h have been suggested by the WHO (Marthaler 1999) for children younger than 6 years, as normal (Table 2.4).

Table 2.4 Suggested normal urine flow rates by the WHO (1999)

WHO	Lower limit	Typical values	Upper limits
Urine volume			
<6 yrs(ml/24h)	140	500	1200
≥ 6 yrs(ml/24h)	200	1200	3000
Urine flow			
<6 yrs(ml/h)	5	20	160
≥ 6yrs (ml/h)	9	50	300

Based on this reference, urine samples with volumes less than 140ml/24h or more than 1200ml/24h, should be discarded.

Several studies have used WHO recommendations to validate 24-h urines (Marthaler *et al.*, 1995, Ketley & Lennon 2000, Ketley & Lennon 2001, Ketley *et al.*, 2004, Maguire *et al.*, 2007). In these studies urine samples with flow rates under or above recommended limits of WHO were excluded, assuming that these samples were either incomplete or diluted by water.

However, it has to be noted that daily urine volume and consequently flow rate is affected by the volume and type of liquid consumption per day.

2.4. Validation of Dietary Assessment Methods

Dietary assessment methods could be associated with random or systematic errors. Random errors affect the precision and could be minimised by increasing the number of observations and subjects. However, systematic errors or "bias" are independent of the number of observations and affect the ability of the method to detect differences in average intake of nutrients between groups of subjects. Several factors could contribute to the systematic error such as the use of food tables, coding errors, failure to report the intake either due to the changes in dietary habits while taking part in the study, misreporting or under-collecting the amount of food and finally reporting the wrong frequency of consumption (Bingham 1987). Various validation studies have been conducted to find the most "accurate" dietary assessment method which is free from both errors. Traditionally validity was based on the comparison between the methods in that investigators validated their method against another method which had greater acceptance if only the estimates of dietary intakes were comparable. However, it never determined if either of the methods was valid. Since all dietary assessment methods rely on subjects giving accurate and truthful reports, no method can be assumed to be valid. Therefore, in the absence of an assessment tool capable of estimating true intake, biomarkers are recognised as the best practice to assess the absolute validity (Bingham 2003). Nutritional biomarkers are used to estimate nutrient intake or compare nutrient intake to that estimated by dietary assessment method. A biological marker could be any biochemical index to be found in an easily accessible biological sample such as urine, blood or hair and closely reflect dietary intake (Bingham 1987).

In addition to biological markers, energy intake as a foundation of diet has been suggested for validating the general quality of the dietary data in any study (Livingstone & Black 2003).

2.4.1. Energy intake (EI) and Physical Activity Level (PAL)

Energy intake of an individual is the amount of dietary energy required to maintain appropriate level of physical activity as well as growth (Torun *et al.*, 1996).

Energy requirements are determined by measurements of energy expenditure and for children an additional allowance for growth (1 to 3%, depending on age) is considered.

Validation of reported energy intake is based on the fundamental equation that energy intake (EI) = energy expenditure (EE) ± changes in body store. In adults at group level the changes in body stores can be ignored assuming weight is stable and therefore, EI is equal to EE (Goldberg *et al.*, 1991). However, due to variation in both measurements, absolute agreement cannot be expected even for valid data (Livingstone & Black 2003). For children younger than 7 years of age dietary energy recommendations are about 20% higher than energy expenditure (Torun *et al.*, 1996).

Previous information on total energy expenditure of children was limited and was mainly based on reported energy intake of well-nourished and healthy children. However, the application of doubly labelled water (DLW), heart rate monitoring (HRM), and time-motion /activity diary (TM) have enabled more accurate measurement of energy expenditure and requirement for children (Torun *et al.*, 1996). The use of DLW for measuring energy expenditure is well established and several studies have used it to validate dietary intakes both in children and adults (Johnson *et al.*, 1996, Black 1996, Black *et al.*, 1996, Bandini *et al.*, 1997, Johnson *et al.*, 1998, Black *et al.*, 2000).

Due to changes in the lifestyle and activity pattern of children, a recent review (Torun 2005) on evaluation of data on total energy expenditure of healthy children aged 1-18 years used DLW, HRM and TM techniques, provided new guidelines for energy requirements of children. The new guidelines indicated lower energy requirements in children compared to what was proposed by FAO/WHO/UNU consultation report (1985) on energy. Estimated Average Requirements (EAR) by

the Department of Health (DH) UK (1997) and reported average EI by the National Diet and Nutritional Survey (NDNS) (Gregory *et al.*, 2000) for 4-6 year old British children are presented in Table 2.5. Reference values of EI for British children by DH include the mean intake which meets average physiological requirements (EAR), while the reported EI by the NDNS includes mean, 2.5 and 97.5 percentile EI.

Due to the high cost of DLW, a relatively simple way to estimate total daily energy expenditure and requirements in adults has also been proposed by the FAO/WHO/UNU (1985) expert consultation group. A factorial calculation accounting for the time allotted to each activity allowed the estimation of the mean PAL in 24h. Therefore, assuming an energy cost of equal to Basal Metabolic Rate (BMR) for sleeping, energy cost for different level of activities was: EI =BMR×PAL (Torun *et al.*, 1996). Depending on activities of different intensities, total energy expenditure or requirements PALs of 1.55, 1.78 and 2.10 for males and 1.26, 1.64 and 1.82 for females was proposed to estimate total energy expenditure or requirement. The same approach was used for children and provisional cut-off points for PAL were suggested for children living in industrialised countries. These cut-off points were 1.28-1.79 for 1-5 year old children of both genders, 1.39- 2.24 and 1.30-2.10 for 6-18 year old boys and girls respectively (Torun *et al.*, 1996). However, these cut-off points can only evaluate bias at the group level and their sensitivities are limited to identify under-reporters at the individual level. Therefore, to identify individual under reporters a detailed questionnaire on physical activity should be obtained from individuals by which a subject-specific PAL could be derived and EI of that subject can then be evaluated at individual level (Torun 2005).

Table 2.5 Reference intake values for energy requirements of children

Age (yr)	Reference daily energy requirements (kcal/d)			Reported average EI in the NDNS (kcal/d) 2000
	Torun (2006)	FAO/WHO/UNU 1985	Department of Health 1997	
Boys 4-6	1252-1467	1560-1810	1600-1810	1520* (914-2126)α
Girls 4-6	1156-1330	1440-1630	1460-1620	1397* (847-1947)α

α lower and upper 2.5 percentile, * Mean value

2.4.2. Twenty-four hour urine nitrogen

2.4.2.1. Nitrogen (protein) function and requirement

Nitrogen is one of the elements for the synthesis of amino-acids which are the major components of proteins. Protein is one of the essential macronutrients for growth and its primary roles include: structural protein, enzymes, hormones transports, and immunoproteins. Protein requirements of various age groups have been measured. For children additional amount of protein which is required for growth was also incorporated into the requirements and reported by FAO/WHO/UNU (FAO/WHO/UNU expert consultation 1985) and the Department of Health (UK) (Department of Health 1997).

2.4.2.2. Studies of nitrogen intake and excretion

Twenty-four hour urine nitrogen is the most well-known biological marker for validation of dietary methods based on the assumption that subjects are healthy and in nitrogen balance and there is no accumulation due to growth or repair of lost muscle tissue or loss due to starvation or injury (Bingham 1994). Several studies have used this biomarker to validate dietary data as summarised in Table 2.6.

The use of 24-h urine nitrogen as an independent validity check in dietary survey methods was first proposed by Isaksson in 1980 (Isaksson 1980). He summarised data on protein intake reported by different dietary surveys: food records, diet history and 24-h recall conducted between 1968 and 1980 on randomly selected populations, groups and individuals. He then compared the average protein intake with protein excretion calculated from single collection of 24-h urines and proposed a formula; protein excretion= 6.25 (U_N +2), where U_N is the 24-h urinary nitrogen in grams and 2 is external excretion representing dermal and faecal losses of nitrogen. Based on their results, Isaksson and co-workers found that 91-98% of dietary nitrogen was excreted in urine when dietary data collected by diet history and food records and therefore these two methods provided more valid data whereas a systematic underreporting was observed in data collected by 24-h recall. The author suggested that daily variation in nitrogen excretion (13%) and intake (21%) as well as the limitation of using single 24-h urine nitrogen for validating nitrogen intakes reported from 24-h recall, could have resulted in underestimation of protein intake (Isaksson 1980).

The value of 24-h urine nitrogen for validation of dietary methods was also examined by other investigators in adults. Bingham and co-workers (Bingham & Cummings 1985) collected diet and 24-h urine samples of eight healthy individuals consuming their normal diets for 28 days. They reported a mean urine to diet nitrogen ratio (UN/DN) of 81% with a range from 78 to 83%. A meta-analysis of a large set of data by Kipnis and co-workers (Kipnis *et al.,* 2001) also confirmed UN/DN ratio of 80% in adults.

In another study by Bingham and co-workers (Bingham *et al.,* 1995) to assess the validity of dietary methods an average UN/DN ratio of 91% with a range from 76 to 113% was reported.

A study on elderly women reported a UN/DN ratio of 118% from diet history method and on the basis of four 24-h urine collections (Visser *et al.,* 1995). The high ratio was attributed to underestimation of protein intake by diet history method or the incomplete urine collections or the age of subjects.

The validity of 4-day food records and duplicate collections was assessed in a group of Swedish women (Johansson *et al.,* 1998). A 126% UN/DN ratio was found for both methods, indicated bias to under-reporting. In a study on 48, 50-60 year old English women compared validity of diet history with diet records the UN/DN ratio was reported to range from 86% to 90% (Black *et al.,* 2000). Twenty-four hour urine samples were collected from 134 subjects over a period of 9 months to compare two dietary methods: "food records" and "FFQ" (McKeown *et al.,* 2001). In that study UN/DN ratios of 93% and 97% from food records and 79% and 91% from FFQ were found in women and men respectively.

Another study on 179 adults reported a mean UN/DN of 82% and 92% by FFQ and food records respectively (Day *et al.,* 2001).

Food records were also validated by using 24-h urine samples and a mean UN/DN ratio of 78% with a range from 66 to 87% was reported (Tasevska *et al.,* 2006).

In summary, from different studies on adults using urinary nitrogen to validate dietary data, UN/DN ratio ranged from 66% to 113%.

The number of studies used urinary nitrogen in children is limited to balance studies for the assessment of protein requirements. Summary of these studies are presented in Table 2.7.

These studies reported nitrogen intake and excretion. However, since no UN/DN ratio was reported from any of these studies, it was attempted to estimate UN/DN ratio using the data on nitrogen intake and excretion.

A series of studies were conducted in early 60's to determine amino acid requirements of 16 healthy Japanese children aged 8-13 years from both genders (Nakagawa *et al.,* 1963, Nakagawa *et al.,* 1965). Daily nitrogen intake and urinary nitrogen excretion for a period of 12-19 days was reported to range from 8.17g/d (girls) to 12.14 g/d (boys) and from 5.19g/d (girls) to 12.30g/d (boys) respectively. The study found a positive balance in the population.

In another study to assess protein requirements of 15, 8 year old Caucasian girls daily nitrogen intake, urine nitrogen as well as nitrogen losses through sweat and faeces was estimated (Howat *et al.,* 1975). A range from 5.4 to 4.12 g/d for intake and from 4.12 to 11.15 g/d for excretion was reported. Based on their data, the UN/DN ratio was estimated at 75% to 80%.

In a balance study on various age groups of children from Iowa, a 0.51 and 0.37 mg/kg bw/d nitrogen intake and excretion was reported for 6 months old. However, the corresponding data was reported at 0.45 and 0.34 mg/kg bw/d for 6 to 11 months old (Ziegler *et al.,* 1977).

A recent study in Germany compared the protein intake estimated by the weighed record with that of estimated from 24-h urine samples (Bokhof *et al.,* 2010). Their results indicated that protein intakes recorded from weighed records was generally less than that of estimated from 24-h urines and the difference increased with age. To estimate protein intake from urine the authors assumed that excretion of nitrogen accounts for 80% of the ingested protein due to extra-renal nitrogen losses (Bingham 2003). Therefore, excreted nitrogen (mmol/L) was converted to gram protein/day [protein g/d= (N g/d × 6.25 ×14)/1000 and then divided by 0.8. From their intake and excretion data, UN/DN ratio was found to range from 103%, to 109% for 3-4, and 11-13 year olds respectively.

Table 2.6 Summary of studies in adults used urinary nitrogen to validate dietary data

Reference	Mean UN/DN (%)	Range UN/DN (%)
Bingham et al (1985)	81	78-83
Van stavern et al (1985)	85	n/a
Visser et al (1995)	118	n/a
Bingham et al (1995())	91	76-113
Johanson et al (1998)	126	n/a
Black et al (2000)	89	86-90
Kipnis et al (2001)	80	n/a
McKeown et al (2001)	93 [F] 97 [M]	n/a
Day et al (2001)	82[∞] 92[δ]	n/a
Tasevska et al (2006)	78	66-87

[∞] Food Frequency Questionnaire

[δ] 7-day record

Table 2.7 Summary of reported mean intake and excretion of nitrogen in balance studies in children

Reference	Age (gender)	Nitrogen intake(g/d)	Nitrogen excretion(g/d)
Nakagawa (1965)	8-13 (F) 10-12 (M)	8.17-10.18 12-14-12.13	5.19-10.81 9.8-12.30
Howat (1975)	8-9.5 (F)	5.44-14.1	4.12-11.15
Ziegler (1977)	3-6 (both) 6-11 (both)	0.51* 0.45*	0.37* 0.34*
Bokhof (2010)	3-4 (both) 7-8 (both) 11-13 (both)	5.6 7.7 9.9	5.8 8.1 11

*based on mg/kg bw/day

2.4.3. Twenty-four hour urine potassium

2.4.3.1. Potassium function and requirement

The main function of potassium in the body is to work closely with sodium to maintain the osmotic pressure within cells. About 98% of total body potassium is

intracellular which is 30 times higher than that of extracellular (Ensminger *et al.*, 1995). Potassium is a cofactor for a number of enzymes, required for secretion of insulin and involved in phosphorylation of creatine in carbohydrate metabolism and protein synthesis (Ensminger *et al.*, 1995).

In children, the potassium content of a new-born infant is 1560 mg/kg body weight. Total body potassium increases sharply during infancy but it would slow down thereafter in line with muscle growth. There is no difference in the amount required between genders before the puberty. However, during puberty potassium intake of boys is greater than girls because of the muscle size (Rodríguez-Soriano 1995). The requirements for potassium has been estimated and reported by the Department of Health (Department of Health 1997) for children up to age 18 and by the NDNS (Gregory *et al.*, 2000) for 4-6 year olds (Table 2.8).

Table 2.8 Daily potassium intake recommended by Department of Health and reported by The National Diet and Nutrition Survey for 4-6 year old British children

Reference	Potassium Intake (g/d)
DH (1997)	
- All	0.600-1.100
NDNS (2000)	
- Boys	1.045-3.019
- Girls	1.016-2.721

2.4.3.2. Renal handling of potassium

Total body potassium depends on the external balance between intake and excretion. The output is mainly regulated by renal excretion. Although urine is the major route of potassium excretion in healthy individuals, faecal excretion of potassium constitutes 11-15% (5-13 mmol/d) of the dietary intake in Western populations (Holbrook *et al.*, 1984, Barlow *et al.*, 1986). A 20% excretion of potassium in faeces has also been reported (Johansson *et al.*, 1998). Therefore it is important to correct urinary potassium excretion for faecal loses while the balance studies are investigated. However, since faecal sampling is associated with major

practical problems and the completeness of markers is rarely validated, most studies assumed a faecal excretion between 11-15% of intake.

Several factors regulate renal excretion of potassium. One is that the excretion quickly adopts potassium intake in that the excretion decreases when intake is low and vice versa which makes it a suitable biomarker for validity check. Another factor is acid-base status. Acute acidosis and /or alkalosis affect the intracellular concentration of potassium which enhances and or reduces the renal excretion of potassium respectively (Stanton & Giebisch 1982).

2.4.3.3. Studies of potassium intake and excretion

Potassium, although less well established than the urine nitrogen, is another biological marker which can be used in addition to urine nitrogen to validate dietary assessment methods. However, the number of studies used this biomarker for validity purpose is limited.

Summary of some of the studies reported urinary potassium excretion and intake is presented in Table 2.9.

The studies in adults reported a urine to diet potassium ratio (UK/DK) between 63% to 103%.

In a study on a group of nine adults to compare sodium and potassium intake with excretion (Schachter *et al.,* 1980), the individual's UK/DK ratios ranged from 76% to 108% and 79% to 129% with the mean of 87% and 99% when dietary data collected by duplicate method and food record respectively.

While in a study on 28 men and women aged 20 to 58 year the UK/DK ratio was reported at 77% with no significant differences between the genders (Holbrook *et al.,* 1984).

The mean UK/DK ratios of 84% and 88% was estimated from data reported by FFQ and 7-day food record in a group of 44 Italian adults (Porrini *et al.,* 1995).

In a study on 13 English adults reported a mean UK/DK ratio at 77%.with a range from 63-88% (Tasevska *et al.,* 2006).

From the studies in children UK/DK was found to range from 36% to 86% (Table 2.9).

Urine to diet potassium ratios of 76% and 63% were estimated from potassium intake and excretion data reported for 5-14 years old children living in Boalusa,

LA, (Voors *et al.,* 1983). While a lower UK/DK ratio of 48% was estimated for 6-15 year old children from Portland, USA (Connor *et al.,* 1984).

In another study on 13-15 year girls from Alabama, USA, UK/DK ranged from 51% to 86% (Clark & Mossholder 1986).

The lowest UK/DK ratio (36%) was estimated for 330, 8 year old Tasmanian children whose population was predominantly white (Jones *et al.,* 2001).

Table 2.9 Summary of studies which have reported urine and dietary potassium

Reference	**Age group**	**Mean UK/DK (%)**	**Range UK/DK (%)**
Mickelsen (1977)	adults	83	79-87
Schachter (1980)	adults	99^{∞} 87^{σ}	$79\text{-}129^{\infty}$ $76\text{-}108^{\sigma}$
Holbrook (1984)	adults	75^{σ} 86^{∞}	n/a
Caggiula (1985)	adults	98	n/a
Deriaze (1991)	adults	73	n/a
Bingham (1994)	adults	90	80-103
Porrini (1995)	adults	88^{*} 84^{δ}	n/a
Bingham (1997)	adults	n/a	68-80
Johanson (1998)	adults	85	n/a
McKeown (2001)	adults	92^{∞} 77^{σ}	n/a
Day (2001)	adults	75^{σ} 89^{*}	n/a
Tasevska (2006)	adults	77	63-88
	Children		
Voors (1983)	5-14	76	n/a
Connor (1984)	10 (±3)	48	n/a
Clark (1986)	13-15	78^{∞} 69^{σ}	$51\text{-}86^{\infty}$ $60\text{-}86^{\sigma}$
Jones (2001)	8	36	n/a

* 7DR, δ FFQ, $^{\infty}$ food records, $^{\sigma}$ analysing food portions

2.5. Fluoride analytical methods

Fluoride in biological and non-biological samples may exist in two forms of: organic covalent fluorine (C-F) and/or inorganic fluoride bound to organic molecules. The latter in biological samples may be present in the form of a) ionic fluoride bound to H^{+} at low pH, b) non-ionic forms bound to metal ions such as Ca^{2+}, Mg^{2+}, Al^{3+}, and macromolecules e.g. proteins in plasma and organic/ mineral sediments in saliva. Covalently bound fluoride within an organic molecule may be divided into three forms of i) acid-labile, ii) alkali-labile, and iii)

acid-alkali labile (Venkateswarlu 1994). However, ionic fluoride is the one of interest in dentistry, medicine and public health.

Determination of fluoride in any sample involves:

a) Releasing bound fluoride ions from complex organic and inorganic matrices, this pre-treatment depends on the nature of the sample and final method used to measure fluoride. The organic fluoride can be converted into inorganic fluoride using different pre-treatments such as open ashing (Taves 1983, Singer & Ophaug 1986a) or using sodium biphenyl reagent to cleave the C-F bonds and releasing fluoride ions (Venkateswarlu 1982).

b) Using a suitable method to separate fluoride from interfering substances such as diffusion, reverse extraction and adsorption of which the diffusion method is the most widely used technique. It was first described by Taves (Taves 1968) for separation of fluoride from plasma and was further modified and adapted by other investigators (Taves 1983, Singer & Ophaug 1986b, Venkateswarlu & Vogel 1996, Soto-Rojas *et al.,* 2005, Martinez-Mier *et al.*, 2011). The advantage of this technique is that fluoride of the original sample quantitatively transferred to a smaller volume consequently the final solution for analysis has a fluoride concentration well within the sensitivity of the fluoride electrode.

c) Obtaining a low fluoride blank which must be significantly smaller than the amount of fluoride involved in the analysis.

d) Final measurement of fluoride. After the treatment and separation, the final fluoride measurement can be done by one of the following ways: spectrophotometry, fluorometry, fluoride ion-specific electrode, gas chromatography and molecular absorption spectrometry.

The use of ion-selective electrode was introduced by Frant and Ross (Frant & Ross 1966) and because of its performance and speed it has been widely adopted as a routine method for measuring ionic fluoride. However, major problem with the fluoride electrode is that it does not respond to non-ionic fluoride and if fluoride is bounded it should be released into ionic form first.

2.6. Fluoride intake studies

Studies of fluoride intake have been conducted in both fluoridated and non-fluoridated areas. Since there are two major sources of fluoride intake: dietary and non-dietary (toothpaste) most of the studies have assessed and reported fluoride intake from both sources and in different age groups (Table 2.10).

Table 2.10 Studies of fluoride intake from diet, toothpaste and total daily intake of children living in fluoridated and non-fluoridated areas

Author (year)	Age (y)	Number of children	Fluoridated area			Non-fluoridated area		
			Diet (mg/d)	Toothpaste (mg/d)	Total (mg/kg bw/d)	Diet (mg/d)	Toothpaste (mg/d)	Total mg/kg bw/d
McClure (USA, 1943)	1-3		0.42-0.83	-	0.026-0.103	-	-	-
Ophaug et al (USA, 1985)	6 mos 2y	44	0.42 0.62	-	0.05 0.05	-	-	-
Schamschula et al (Hungary, 1988)	3.9	18 28 21	0.72 1.11$^{\theta}$	-	-	0.22	-	-
Burt (USA, 1992)	1-3 3-6		0.65 0.90	-	0.04-0.07 0.03-0.05	-	-	-
Chowdhury et al (New Zealand,1996)	3-4	66	0.36	0.32	0.036	0.15	0.34	0.027
Rojas-Sanchez et al. (USA,1999)	1.3-3.3	29 11 14	0.542 - -	0.424 - -	0.07 - -	- 0.219 0.389	- 0.548 0.576	- 0.056 0.073

Continued Table 2.10

Author (year)	Age (y)	Number of children	Fluoridated area			Non-fluoridated area		
			Diet mg/d	Toothpaste mg/d	Total mg/kg bw/d	Diet mg/d	Toothpaste mg/d	Total mg/kgbw/d
Villa et al (Chile, 2000)	3-5	20	0.765	0.254	0.064	-	-	-
Zohouri et al (Iran, 2000)	4	10 8 28 32	-	-	-	0.575 0.440 0.364 0.318	0 0 0.011 0.104	0.039 0.029 0.028 0.030
Kimura et al (2001)	1-6	29	-	-	-	0.28	-	0.019
Haftenberger (Germany, 2001)	3-6	11	0.202	0.274	0.053	-	-	-
Grijalva et al (Mexico, 2001)	8-9	10 10 11	5.41[a] 2.32[e] 1.51[x]	-	-	-	-	-
Murakami et al (Japan, 2002)	3-5	93				0.29	0.06	0.021
Martinez-Mier (Mexico, 2003)	1.2-3	19 21	0.696 0.633	0.909 0.971	0.180 0.200	-	-	-
Pessan et al (Brazil,2003)	4-7	21	0.398	0.767	0.056	-	-	-
Paiva et al (Brazil, 2003)	1.6-3.2	32 39	0.027[*] 0.040[*]	0.061[*] 0.052[*]	0.088 0.090	-	-	-
Franco et al (Colombia, 2005)	1.8-2.1	118	-	-	-	n/r 0.028[*α] 0.036[*β]	n/r 0.045[*α] 0.107[*β]	0.110[ə] 0.07[α] 0.14[β]

Continued Table 2.10

Author (year)	Age (y)	Number of children	Fluoridated area			Non-fluoridated area		
			Diet mg/d	Toothpaste mg/d	Total mg/kg bw/d	Diet mg/d	Toothpaste mg/d	Total mg/kgbw/d
Nohno et al (Japan, 2006)	2-5 6-8	21 17	0.025*γ 0.025*	-	-	0.013 0.014	-	-
de Almeida et al (Brazil, 2007)	1-3	33	0.31	1.75	0.130	-	-	-
Maguire et al (UK, 2007)	6-7	6 9 18	0.565δ 0.349σ	0.478 0.534	0.047 0.038	0.188	0.549	0.031
Rodrigues et al (Brazil, Peru, 2009)	26	4-6	0.04* 0.06* 0.05* 0.06*	-	-	0.01*	-	-
Miziara et al (2009, Brazil)	379	2-6	0.478	0.614	0.064	-	-	-

θ F concentration 1.6-3.1 mg/l
b Boys
g Girls
ə overall
*Based on body weight
α High socio-economic area
β Low socio-economic area
γ F concentration at 0.55 mg/l
δ F concentration >0.7 mg/l
σ F concentration ≥0.3 to <0.7 mg/l
a F concentration at 2.77 mg/l
c F concentration 0.78 mg/l
x F concentration 0.54 mg/l

2.6.1. Fluoride intake from diet

The two major dietary sources of fluoride intake are drinks and food of which drinks can be divided into drinking water and other drinks (excluding water). There might be considerable variation in the fluoride contents of food and drinks due to variation in the level of fluoride in water as well as the amount of water used for preparation and or cooking (Singer & Ophaug 1979, Adair & Wei 1979, Ismail *et al.*, 1984, Clovis & Hargreaves 1988).

Water by itself as a drink or added to other drinks and foods during preparation and cooking could be a major source of fluoride intake in some children living in fluoridated areas. However, fluoride ingestion from water can be influenced by: 1) fluoride concentration of water, 2) age of the individuals, 3) climate conditions, and 4) dietary habits (Murray 1986b).

Drinking water contributed to 30% of dietary fluoride intake in 1-10 year old US children (Ershow & Cantor 1989). While for 8-9 year old Mexican children living in fluoridated area (2.77 mg/l) a contribution of 63% was reported from water (Grijalva-Haro *et al.,* 2001).

For 6-7 year old British children water was not found to be predominant source of fluoride intake and contributed to 11% and 24% for children living in optimally and sub-optimally fluoridated areas respectively (Zohouri *et al.,* 2006).

Recent reports show a decline in water consumption but an increase in beverage consumption over the past 25 years (Ismail *et al.,* 1984, Clovis & Hargreaves 1988). The reported contribution of drinks to total dietary fluoride intake ranged from 7% in 6-7 year old British children living in non-fluoridated areas (Maguire *et al.,* 2007) to 72% in 3-6 year old German children who received fluoridated salt (Haftenberger *et al.,* 2001).

The contribution of food to total daily dietary fluoride intake reported to range from 27% in 16-40 months old US children (Rojas-Sanchez *et al.,* 1999) to 84% in 15-36 months old Mexican children (Martinez-Mier *et al.,* 2003). Among the food, those which are cooked with water have been reported to contribute to daily dietary fluoride intake substantially. While in 4 year old Hungarian children the 53% contribution of food to daily dietary fluoride intake was mostly from soups (Schamschula *et al.,* 1988), in 6-7 year old British children 16% of fluoride intake from food was contributed by the food made with water such as rice and pasta (Zohouri *et al.,* 2006).

Overall, the contribution of dietary sources varies among different populations and depends on dietary habits and climate temperature.

The highest reported dietary fluoride intake from both food and drinks (including water) was for 8-9 year old Mexican children (5.41 mg/d) with 3mg F/l in their drinking water (Grijalva-Haro *et al.,* 2001) followed by 1.11 mg/d for 4 year old Hungarian children with fluoridated water of 1.6-3 mg/l (Schamschula *et al.,* 1988). Both groups were from areas with high fluoride concentration in their drinking water.

However, in optimally fluoridated areas reported dietary fluoride intakes ranged from 0.202 mg/d in 3-6 year old German children (Haftenberger *et al.,* 2001) to 2.32 in 8-9 year old Mexican children (Grijalva-Haro *et al.,* 2001).

Dietary fluoride intake of 1-3 year old US (0.83mg/d) (McClure 1943) and Mexican children (0.70 mg/d) (Martinez-Mier *et al.,* 2003), 3-5 year old Chilean (0.76 mg/d) (Villa *et al.,* 2000) and 4 year old Hungarian children (0.72 mg/d) (Schamschula *et al.,* 1988) were also found to be high.

Among children living in low or non-fluoridated areas dietary fluoride intake ranged from 0.15 mg/d in 3-4 year old children from New Zealand (Guha-Chowdhury *et al.,* 1996) to 0.575 mg/d in 4 year old Iranian children (Zohouri & Rugg-Gunn 2000b).

A mean dietary fluoride intake from 0.04 to 0.06 mg/kg bw/d was reported for 4-6 year old Peruvian children whose fluoride sources were fluoridated salt and milk (Rodrigues *et al.,* 2009).

2.6.2. Fluoride intake from toothpaste

It is generally accepted that the widespread use of fluoridated toothpaste has had an important effect in reducing dental caries prevalence among children and young adults in many western countries (Fejerskov *et al.,* 1982). Although, fluoridated toothpastes intended for topical use, they can inadvertently be ingested and consequently be a major source of fluoride intake in some children. Some studies on fluoride absorption from ingested toothpaste concluded that the absorption is close to 100%, all of which is readily bioavailable (Ekstrand & Ehrnebo 1980). Therefore fluoridated toothpastes during the "critical period" of tooth development (birth to 6 years) can be a risk factor for fluorosis (Osuji 1988, Lalumandier 1992). The susceptibility of the permanent incisors and the first permanent molars to fluorosis would appear to be greatest during the first 4 years of life (Bardsen *et al.,* 1999), as these teeth begin to form soon after birth and erupt at about 7 years of age. However, chronic excessive intake of systemic

fluoride from 3 to 6 years of age can also put the later erupting permanent canines, premolars and second molars at risk (Levy 2003).

Children under the age of 6 years might swallow between 25% to 65% of the toothpaste at each brushing episode which is mainly attributed to their inability to control their swallowing reflexes (Ripa 1987, Naccache *et al.,* 1992). Other factors that strengthen the likelihood of fluoride toothpaste being a risk factor for fluorosis are: i) early start of brushing with fluoridated toothpaste before the age of 2 (Osuji 1988, Levy & Zarei-M 1991), ii) unsupervised brushing (Dowell 1981, Levy & Zarei-M 1991), and iii) use of large amount of toothpaste (Dowell 1981, Levy *et al.,* 1993). A study on 2-7 year old Canadian children reported a higher toothpaste usage among 2-year olds (0.6g) compared with 3, 4 and 5 year olds who used 0.5, 0.4 and 0.5 g respectively (Naccache *et al.,* 1992). In addition, other tooth brushing habits such as the frequency of brushing (Pendrys 1995) and the fluoride concentration of toothpastes (Rock 1994) have been reported to contribute to greater fluoride intake from toothpastes and subsequently the risk of developing dental fluorosis.

In the UK, it was reported that 46% of children start brushing by the age of 12 months and 74% start to use toothpaste by the age of 18 months (Dowell 1981). While in the United State 56% of children reported to start brushing at 9 months, of whom 30% used fluoridated toothpaste (Levy *et al.,* 1995).

The contribution of fluoridated toothpaste to total daily fluoride intake has been reported to range from 25% in 4 year old Iranian children (Zohouri & Rugg-Gunn 2000b) to 72% in 16-40 and 15-36 month old US and Mexican children (Rojas-Sanchez *et al.,* 1999, Martinez-Mier *et al.,* 2003).

In children younger than 4 years of age, the mean quantity of fluoride ingested has been found to range from 0.254 (Villa *et al.,* 2000) to 1.75 mg/d (de Almeida *et al.,* 2007). Fluoride intake from toothpastes in 16-40 months old children living in non-fluoridated San Juan and Connersville were found to be high at 0.767 and 0.965 mg/d respectively while children from fluoridated Indianapolis had lower fluoride intake from this source, (0.424 mg/d) (Rojas-Sanchez *et al.,* 1999).

A high fluoride intake from toothpaste was reported for 15-36 months old Mexican children at 0.908 and 0.971 mg/d living in Veracruz and Mexico City respectively (Martinez-Mier *et al.,* 2003).

For 22-35 months old Colombian children fluoride intake from toothpaste was higher among low socio-economic areas (0.107 mg/kg bw/d) compared with that of in high socio-economic areas (0.045 mg/kg bw/d) (Franco *et al.,* 2005a).

For 4 to 6 year old children toothpaste ingestion ranged from 0.011 (Zohouri & Rugg-Gunn 2000b) to 0.767 mg/day (Pessan *et al.,* 2003) while for 6-7 years old this range was narrower from 0.478 to 0.534 mg/d (Maguire *et al.,* 2007).

Brushing activities including rinsing and expectoration have been reported to be associated with fluoride ingestion from toothpaste in several studies (Baxter 1980, Naccache *et al.,* 1992, Sjögren *et al.,* 1994). In a recent study on 155, 2-6 year old Brazilian children a significant difference was reported in the percentage of fluoride ingested among different ages, different toothpaste flavours and toothpaste amounts used (Kobayashi *et al.,* 2011).

2.6.3. Total fluoride intake

The focus of this section is on studies in which total fluoride intake from diet and toothpaste in both fluoridated and non-fluoridated areas has been reported.

- **Non-fluoridated areas**

A total daily fluoride intake of 0.027 mg/kg bw/d has been reported for 3-4 year old children living in non-fluoridated New Zealand (Guha-Chowdhury *et al.,* 1996) which was similar to that of 4 year old Iranian children (0.03 mg/kg bw/d) (Zohouri & Rugg-Gunn 2000b) and 6-7 year old British children (0.031 mg/kg bw/d) (Maguire *et al.,* 2007). However, it was lower than figures of 0.056 and 0.073 mg/kg bw/d for 16-40 month old US children living in two non-fluoridated communities (Rojas-Sanchez *et al.,* 1999).

A total daily fluoride intake of 0.02 mg/kg bw/d has been reported for 2-5 year old Japanese children living in non-fluoridated areas (Murakami *et al.,* 2002).

The mean total daily fluoride intake of 22-35 months old Colombian children was found to be high at 0.11 mg/kg bw/d (Franco *et al.,* 2005a). For Colombian children also a significantly higher total daily fluoride intake was reported in children from low socio-economic status (0.14 mg/kg bw/d) compared with (0.07 mg/kg bw/d) that of children from high socio-economic areas.

- **Fluoridated area**

A wide range from 0.026 to 0.103 mg/kg bw/d was reported for total daily fluoride intake of 1-3 year old US children (McClure 1943) which was close to ranges of 0.03

to 0.07 mg/kg bw/d reported for 1-6 year old US children (Burt 1992). Mean total daily fluoride intake of 0.05 mg/kg bw/d reported for 6 months and 2 year old US children (Ophaug *et al.,* 1985) was similar to those of for 3-6 year old German children (0.053 mg/kg bw/d) (Haftenberger *et al.,* 2001) and 6-7 year old British children (0.047 mg/kg bw/d) (Maguire *et al.,* 2007) but it was lower than 0.2 mg/kg bw/d reported for 15-36 month old Mexican children (Martinez-Mier *et al.,* 2003). It has to be noted that both German and Mexican children received fluoridated salt.

A mean total daily fluoride intake of 0.09 mg/kg bw/d reported for 19-38 months old Brazilian children (Paiva *et al.,* 2003) which was higher than reported values of 0.07 mg/kg bw/d for 16-40 month old US (Rojas-Sanchez *et al.,* 1999), 0.064 mg/kg bw/d for 3-5 and 2-6 year old Chilean (Villa *et al.,* 2000) and Brazilian, (Miziara *et al.,* 2009) and 0.056 mg/kg bw/d for 4-7 year old Brazilian children (Pessan *et al.,* 2003).

Mean total daily fluoride intake of 0.036 mg/kg bw/d reported for 3-4 year old children from New Zealand (Guha-Chowdhury *et al.,* 1996) was similar to that of for 6-7 year old British children (0.038 mg/kg bw/d) (Maguire *et al.,* 2007). It has to be addressed that this group of British children received sub-optimal fluoridated water ($\geq$0.3 to <0.7 mg/l).

The uppermost limit of total fluoride intake from "all sources" including dietary (food and beverages), non-dietary (toothpastes, mouthrinses and gels) and fluoride supplements has been estimated between 0.05 to 0.07 mg/kg bw/day (Burt 1992). However, it has been argued that the threshold level of fluoride intake for the development of dental fluorosis may be as low as 0.03-0.04 mg/kgbw/day (Baelum *et al.,* 1987, Fejerskov *et al.,* 1987).

While the mean total daily fluoride intake for most of children in the above studies was within the optimal range , it exceeded the upper limit of optimal range of 0.07 mg/kg bw/d for Mexican (Martinez-Mier *et al.,* 2003) and Brazilian children (Paiva *et al.,* 2003, de Almeida *et al.,* 2007).

2.6.4. Incidence of dental fluorosis in fluoridated and non-fluoridated areas

The incidence of dental fluorosis is related to chronic fluoride exposure during enamel development and its severity is influenced by the dose, timing and duration of fluoride exposure (Browne *et al.,* 2005). Children living in both fluoridated and non-fluoridated areas are exposed to different fluoride sources since early childhood. In order to prevent dental fluorosis it is important to assess fluoride intake from main

sources such as diet and toothpaste as well as the contribution of each source. There have been some reports on the prevalence of dental fluorosis in both fluoridated and non-fluoridated areas. In Ireland, the proportion of 5 year old children with enamel fluorosis in their primary teeth was reported at 29.4% and 1.2% in fluoridated and non-fluoridated communities respectively (Harding *et al.,* 2003). In the UK the prevalence of fluorosis in 8-9 year old children living in fluoridated communities reported at 54% compared with 23% living in non-fluoridated area. (Tabari *et al.*, 2000).

2.7. Fluoride excretion studies

The rate of urinary fluoride excretion varies throughout the day and night in relation to the time of fluoride ingestion. Therefore, period of urine collection should ideally cover as much of the 24-h period as possible. The 24-h urine has been regarded as a reliable period of time for urine collection which is independent of dietary habits, timing of meals and periods of maximal fluoride intake (Marthaler 1999). However, the collection of 24-h urine samples may not be a convenient method for monitoring fluoride excretion of large groups within the community (Lennon *et al.,* 1996).

Therefore, some studies employed the supervised timed urinary collection mainly due to difficulties in collecting 24-h urine.

Due to difficulties in measuring fluoride intake in children, urinary fluoride excretion has been suggested as a useful indication of fluoride exposure. A range of provisional standards has been established by the WHO (1999) for the urinary fluoride excretion of 3-14 year old children receiving low and or optimum fluoride (Table 2.11).

Urinary excretion studies have been carried out during fluoridation programs of salt (Warpeha & Marthaler 1995, Marthaler *et al.,* 2000), milk (Ketley & Lennon 2000, Villa *et al.,* 2000, Ketley & Lennon 2001) and water (Rugg-Gunn *et al.,* 1993) and to study the additional influence of using fluoridated toothpaste (Udipi *et al.,* 1993, Ketley *et al.,* 2004). Summary of studies on urinary fluoride excretion are presented in Table 2.12.

- **Fluoridated area**

Urinary fluoride excretion of children living in fluoridated areas reported to range from 0.229 mg/d in 3-5 year old Chilean children with a water fluoride concentration of 0.6 mg/l (Villa *et al.,* 1999) to 3.100 mg/d in 8-9 year old children from Bumbillias, Mexico with a water fluoride concentration of 2.77 mg/l (Grijalva-Haro *et*

al., 2001). Urinary fluoride excretion of 0.229 mg/d was lower for 3-5 year old Chilean children when fluoridated water (0.6 mg/l) was the only source of fluoride compared with an excretion of 0.526 mg/d when fluoride was added to juices and given to children as an additional source of fluoride intake to the water (Villa *et al.,* 1999). The fluoride excretion of German children aged 3-6 years, living in non-fluoridated water but receiving fluoridated salt (Haftenberger *et al.,* 2001) was found to be close to that of 4 year old English children who received optimally fluoridated water (Rugg-Gunn *et al.,* 1993).

- **Low /non-fluoridated areas**

Urinary fluoride excretion of children living in low or non-fluoridated areas was reported to range from 0.136 mg/d for 4 year old Venezuelan children who received low fluoridated water (0.1 mg/l) and salt (60 mg/kg) (Acevedo *et al.,* 2007) to 0.339 mg/d for 4 year old Iranian who received low fluoridated water (0.3 mg/l) (Zohouri & Rugg-Gunn 2000b).

A range from 0.170 mg/d to 0.330 mg/d was reported for children aged 1.5 to 3.5 years living in Iceland and Portugal respectively receiving water fluoride concentration <0.15 mg F/l (Ketley *et al.,* 2004).

Mean urinary fluoride excretion of 4 and 5 year old Venezuelan children at 0.203 and 0.207 mg/d with low fluoridated water (0.3 and 0.1 mg/l) and salt (60 mg/l) (Acevedo *et al.,* 2007) was found to be similar to those reported for 6-7 year old British children living in non-fluoridated areas (Maguire *et al.,* 2007).

Table 2.11 Provisional standards recommended by WHO (1999) for 24-h urinary fluoride excretion (UFE) of different age groups

24h UFE	Lower (mg F)	Upper (mg F)
Age 3-5y		
Low F intake	0.17	0.29
Optimal F usage	0.36	0.48
Age 6-7 y		
Low F intake	0.19	0.31
Optimal F usage	0.48	0.60
Age 10-14y		
Low F intake	0.22	0.34
Optimal F usage	0.60	0.82

Table 2.12 Studies of urinary fluoride excretion in optimally and non/low fluoridated areas

Author (year)	Country	Age (y)	N	24 h urinary F excretion		Source of fluoride
				mg/d	mg/kg bw/d	
Rug-Gunn et al. (1993)	Uk Sri-Lanka	4	44 53	0.420 0.550	n/r n/r	 DW (1mg/l)
Villa et al. (1999)	Chile	3-5	42 46	0.229 0.526	0.015 0.028	DW (0.57-0.62 mg/l) DW (0.57-0.62) +F supplement (1mg in 50 ml orange juice)
Zohouri and Rug-Gunn (2000)	Iran	4	78	0.339	0.024	DW (0.33mg/l)
Ketley and Lennon (2000)	UK	4-5	8	0.330	0.017	Milk (0.5 mg)
Baez et al (2000)*	USA	4-6	31	0.750	0.042	DW (1.0-1.3 mg/l) at school Household water from 0.1-3.2 mg/l
Villa et al. (2000)	Chile	3-5	20	0.358	0.022	DW (0.5-0.6 mg/l)
Haftenberger et al. (2001)	Germany	3-6	11	0.476	0.026	F salt
Grijalva-Haro et al. (2001)	Mexico	8-9	11 10 11	0.930 1.040 3.100	0.034 0.038 0.115	DW (0.54 mg/l) DW (0.78 mg/l) DW (2.77 mg/l)
Villa et al. (2002)	Chile	6-8	26	0.387	0.018	Milk (0.625mg)
Ketley et al (2004)	Ireland England Finland Iceland Netherland Potrtugal	3	19 18 18 4 6 21	0.370 0.200 0.160 0.170 0.210 0.330	0.022 0.014 0.011 0.011 0.014 0.022	DW (0.8-1.0 mg/l) DW (<0.15 mg/l) DW (<0.15 mg/l) DW (<0.15 mg/l) DW (<0.15 mg/l) DW (<0.15 mg/l)

Continued Table 2.12

Author (year)	Country	Age (y)	N	24 h urinary F excretion		Source of fluoride
				mg/d	mg/kg bw/d	
Maguire et al (2007)	UK	6-7	18	0.203	0.008	DW (0.08 mg/l)
			8	0.239	0.011	DW (0.47 mg/l)
			3	0.323	0.014	DW (0.8 mg/l)
Acevedo et al. (2007)	Venezuela	3	8	0.188	0.013	DW (0.12 mg/l)+salt (60-90 mg/kg)
			10	0.273	0.019	DW (0.34 mg/l) +salt (60-90 mg/kg)
		4	11	0.136	0.009	DW (0.12 mg/l)+salt (60-90 mg/kg)
			11	0.203	0.013	DW (0.34 mg/l) +salt (60-90 mg/kg)
		5	12	0.207	0.011	DW (0.12 mg/l)+salt (60-90 mg/kg)
			8	0.287	0.016	DW (0.34 mg/l) +salt (60-90 mg/kg)

*based on 15 hours urine collection

2.8. Fluoride intake/excretion studies

- **Fractional Urinary Fluoride Excretion**

Monitoring fluoride exposure is an important part of community-based fluoridation programmes (Marthaler 1999) which has been mainly conducted through urinary fluoride excretion. However, to estimate fluoride ingestion from excretion data, it is essential to establish the value of the fraction of total daily fluoride intake that is excreted through urine under customary conditions of fluoride intake in any age group (Villa *et al.,* 2000).

A few studies have measured both total fluoride intake and urinary fluoride excretion in the same children.

A fractional urinary fluoride excretion of 85% and 80% was reported for 3-4 year old US (Brunetti & Newbrun 1983) and 4 year old Iranian children (Zohouri & Rugg-Gunn 2000b) living in optimally and low fluoride areas respectively. The corresponding value was 40% for formula-fed infants receiving water fluoride concentration of 1mg/l (Ekstrand *et al.,* 1984), 35.5% for 3 to 5 years old Chilean children living in optimally fluoridated area (Villa *et al.,* 2000) and 51.5% for 3-6 year old German children receiving fluoridated salt (Haftenberger *et al.,* 2001).

A fractional urinary fluoride excretion of 61%, 45% and 57% was also reported for 8-9 year old Mexican children living in communities with 0.54, 0.78 and 2.77 mg F/l in the water supply respectively (Grijalva-Haro *et al.,* 2001).

In a more recent study fractional urinary fluoride excretions of 32%, 40% and 44% was reported for 6-7 year old children living in optimally (0.82 mg/l), sub-optimally (0.47 mg/l) and non-fluoridated areas (0.08 mg/l) respectively (Maguire *et al.,* 2007).

- **Fluoride retention**

Almost 10% of daily ingested fluoride is excreted in faeces (Ekstrand & Whitford 1996). Therefore, fluoride retention is usually calculated by subtracting total fluoride excretion through urine and faeces from total daily fluoride intake.

Original studies by Machle and Largent (Machle *et al.,* 1942) suggested that fluoride ingestion can be estimated by doubling fluoride excretion data.

However, although this assumption may be valid for adults, it is questionable for growing children. During skeletal growth a relatively high proportion of ingested fluoride is deposited in the skeleton. Therefore in very young children a larger

fraction of a single dose will be retained in skeleton compared with the same dose given to an adult (Ekstrand & Whitford 1996). However, among the children the rates of fluoride retention differ which can be influenced by some dietary factors. Divalent or trivalent cations such as Calcium and Magnesium or aluminium can form insoluble complex with fluoride which significantly reduces fluoride absorption, while dietary protein and fat increases fluoride absorption (Cerklewski 1997).

It has been suggested that 70% of ingested fluoride is retained by pre-school children (Murray 1986b). However, the reported percentage for 4 year old Iranian children (Zohouri & Rugg-Gunn 2000b) was less than 20%. A lower proportion of 12.5% was reported in a fluoride balance study on 11 breast-fed infants (Ekstrand 1994). The study on 3-5 year old Chilean children reported that 55% of the total daily fluoride intake was retained in the body (Villa *et al.,* 2000).

The study in 6-7 year old British children (Maguire *et al.,* 2007) reported a strong positive correlation between total daily fluoride intake and fluoride retention. The proportion of fluoride retained in these children reported at 58%, 50% and 46% for children living in optimally, sub-optimally and non-fluoridated areas respectively. However, the authors did not find any correlation between fluoride retention and fluoride concentration of tap water. The difference in the reported values can be attributed to i) the difference in the age of the study groups, ii) type of diet, iii) method of data collection and iv) fluoride concentration of tap water.

In younger children there is a higher rate of fluoride deposition in calcified tissues compared with older children. A vegetarian diet can also increase the pH of the tubular fluid which is associated with higher fluoride excretion. In addition, different methods such as food diary and duplicate plate methods have been used to collect dietary data. Finally the difference in fluoride concentration of tap water among the study groups could have resulted in the differences in fluoride intake and consequently retention.

2.9. Summary and conclusion

In view of the narrow margin between optimal and excessive fluoride intake/exposure, considerable interest has recently been focused on studies involving measurement of fluoride intake and retention. Different approaches have been used to estimate dietary fluoride intake including: market basket

collection, food frequency questionnaire, 3-day dietary diary and 1- or 2-day duplicate plate collection. However, 3-day food diary and 2-day duplicate plate methods are the most frequently used techniques for estimating fluoride intake in children and adults.

Nevertheless, neither of these two methods have been validated and evaluated for their abilities to provide less under-reporting or over-reporting dietary data. In the absence of standardised universal method for the assessment of fluoride intake, comparing the results between studies is difficult since it is not clear whether there are true variations in fluoride exposure or the difference is due to the dissimilarity in the method. Besides, to conduct studies on fluoride intake and excretion, it must be first determined which method of recording food and beverage intake is likely to provide more accurate data.

Hence, a universal dietary fluoride assessment tool is needed for epidemiologists and health researchers to estimate dietary fluoride intake and investigate the relationships between fluoride intake and human health.

On the other hand, the accurate assessment of food intake in children is challenging. Current available dietary methods which were constructed for use in adults have also been used in children. However, the choice of dietary method for a population depends on the objectives of the study, the number, and characteristics of the study population.

The literature showed that nutritional biomarkers (nitrogen and potassium) have been used as independent validity checks in studies with adults while information regarding the use of these biomarkers for validation of dietary intakes in children was limited. However, urine nitrogen and potassium have been measured in balance studies and those related to hypertension or bone density in children.

It has to be noted that the use of urinary biomarkers as independent validity checks in dietary surveys can be inaccurate if the measures to verify the completeness of 24-h urine collections are absent. Creatinine excretion and urinary flow rates have been suggested as assessment tools for checking the completeness of 24-h urine collections.

From the reference ranges available in the literature for creatinine excretion the range of 8-22 mg/kg bw/d suggested for children of all age groups except infants was found to provide more comprehensive basis for checking the completeness of 24-h urine samples.

In addition the availability of multiple sources of fluoride including dietary and non-dietary sources, make the assessment of fluoride intake more complicated. Therefore, many investigators attempted to investigate fluoride intake from other sources such as toothpaste as well as diet. However, the finding that toothpaste contributes to substantial proportion of total fluoride intake is common in most of the studies.

Urinary fluoride excretions have been used to estimate total fluoride intake. Various studies have attempted to quantify fractional fluoride excretion in young children. However, the proposed values in the literature are conflicting and further studies in both intake and excretion are required to establish these values for different age groups.

Therefore, data on total daily fluoride intake and excretion on a larger group of young children may provide an inclusive basis for future fluoride exposure studies aiming to use urinary fluoride excretion as a marker of fluoride intake.

Chapter 3 Aim

3.1. Main Aim

The main aims of this study were to develop a better understanding of strengths and weaknesses of the dietary methods "2-day duplicate" and "3-day food diary" by comparing dietary fluoride intake estimated by each method and evaluate the validity of the two methods for estimating dietary fluoride intake in young children.

3.2. Subsidiary aims

- Evaluate the response and completion rates among deprived and affluent social groups
- measure total daily fluoride intake (from diet and toothpaste ingestion) in 4-6 year old children residing in a fluoridated area of north-east England, by each method at the group and individual levels
- identify main sources of fluoride intake in children
- measure fluoride excretion in children
- estimate daily fractional urinary fluoride excretion
- estimate total daily fluoride retention in children
- estimate daily fractional fluoride retention in children
- evaluate the correlation between total daily fluoride intake and i) daily fluoride excretion, ii) fractional urinary fluoride excretion, iii) daily fluoride retention, and iv) fractional fluoride retention in 4-6 year old children residing in a fluoridated area of north-east England.
- investigate within and between child variability in dietary fluoride intake, fluoride ingestion from toothpaste and urinary fluoride excretion.

Chapter 4 Pilot investigation on development of an analytical method for measurement of fluoride in expectorated saliva/toothpaste

4.1. Introduction

There are several techniques for the separation and measurement of fluoride in toothpaste. Toothpaste may contain (i) fluoride ions (F^-), (ii) soluble complex fluorides such as FPO_3^{2-}, PF_6^-, BF_4^- which are stable in aqueous solution, and (iii) fluorine compounds which are not water soluble. Over 95% of commercially available toothpastes contain fluorine compounds such as sodium fluoride (NaF), sodium monofluorophosphate (Na_2FPO_3- SMFP), stannous fluoride (SnF_2) and amine fluoride (Hattab 1989).

The first step in measuring fluoride content of a toothpaste is to separate the ionic fluoride from interfering agents followed by measurement of the separated fluoride using either an F ion-selective electrode or gas chromatography.

Light and Cappucino (Light & Cappuccino 1975) reported that by adding a large amount of Total Ionic Strength Adjustment Buffer (TISAB) to toothpaste slurry, almost all fluorides in NaF and SnF_2 toothpastes will exist in ionic form. These are detectable by an F ion-selective electrode. Ionic fluoride in NaF toothpaste can also be determined by adding sodium acetate buffers to the supernatant of toothpaste slurries followed by determination using an F ion-selective electrode (Hattab 1989). However, the SMFP toothpastes should first be hydrolysed to fluoride and orthophosphate using either acids such as perchloric and hydrochloric acid (Grøn *et al.*, 1971) or by using phosphatase enzyme (Duckworth *et al.*, 1991). A fluoride electrode might be employed to measure free fluoride before and after hydrolysis of SMFP. Ashing and distillation of samples followed by measurement with a spectrophotometer or fluoride electrode may also be employed to estimate total fluoride in toothpaste (Lindahl 1983). The hexamethyldisiloxane (HMDS) acid-diffusion method has also been used in some studies to measure total fluoride concentration of toothpaste (Hattab 1989).

In children, brushing the teeth with toothpaste is the most accepted method of tooth cleaning. A marked reduction in caries prevalence has been found as a result of regular use of fluoridated toothpaste. On the other hand, ingestion of fluoride containing toothpaste during tooth brushing by children may be the main

contributor to the increased prevalence of dental fluorosis. Therefore, fluoride ingestion during tooth brushing has been estimated as a part of fluoride exposure studies in children from different countries. However, the amount of fluoride ingested during brushing between studies may not be comparable due to differences in the analytical methods used to measure fluoride in expectorated saliva samples.

Most common types of children's toothpaste contain fluoride in the form of either NaF or SMFP or a combination of both. Therefore, measurement of fluoride in expectorated saliva/toothpaste should be similar to the methods used for fluoride measurement of toothpaste samples. To measure fluoride concentration of expectorated saliva, several studies (Rojas-Sanchez *et al.,* 1999, Martinez-Mier *et al.,* 2003, Pessan *et al.,* 2003, Franco *et al.,* 2005a) employed the HMDS acid-diffusion method, which is the most common method for fluoride analysis of food samples. In this method, sample preparation and fluoride analysis are the same for all sample types regardless of whether the dispensed toothpaste contained fluoride in the form of NaF or SMFP. However, some other studies measured fluoride concentration of the samples directly using the F ion-selective electrode method with or without some kind of pre-treatment depending on the type of fluoride. Centrifugation and/or filtration was used by some investigators to separate out possible dislodged food particles, plaque, and other fine particulates that may interact with fluoride ions once released from the monofluorophosphate, such as the calcium carbonate and dicalcium phosphate dihydrate (DCPD) abrasives which are often used in SMFP pastes.

Chen and co-workers (2006) suggested centrifuging samples for 10 minutes at 930g at room temperature (23°C) to remove particulates. Whereas, Cochran (Cochran *et al.,* 2004b) suggested vortexing samples for 5 minutes followed by either direct measurement for samples containing NaF or hydrolysing samples containing SMFP for at least 3h at 37°C using phosphatase enzyme. They filtered paste extracts but not saliva expectorates prior to fluoride analysis, whereas van Loveren (Van Loveren *et al.,* 2004) filtered all samples.

4.2. Aims

The aims of this pilot study were to find a simple, rapid and accurate technique for preparation and analysis of i) toothpaste samples and ii) expectorated saliva prior to measuring fluoride concentration with the F ion-selective electrode.

4.3. Materials and methods

4.3.1. Preparation and analysis of toothpaste samples

Three toothpaste brands containing different forms of fluoride were chosen from those sold in the UK market: Aquafresh, big teeth (NaF, 1400 ppm), Superdrug (SMFP, 1000 ppm) and Signal, family protection (NaF and SMFP, 1450 ppm).
Before analysis 2 cm of each toothpaste was discarded then one gram from each toothpastes was measured, added to 10 ml diH_2O (1:10) and stirred for 5 minutes. The slurry was then further diluted by adding 10g of diH_2O to 0.1g slurry (1:100) (Cochran *et al.*, 2004b).
Toothpastes containing SMFP were treated with acid buffer and acid phosphatase and incubated for 3 hours (Duckworth *et al.*, 1991).
The fluoride concentration of treated toothpaste samples was measured using the F ion-selective electrode after adding TISAB III. The percentage difference between measured and labelled fluoride concentrations was obtained for all types of toothpastes.

4.3.2. Preparation and analysis of expectorated saliva, toothpaste and rinse

Three adult volunteers aged 25-50 were asked to brush their teeth with two types of toothpastes containing: 1) NaF (Aquafresh, big teeth, 1400 ppm), and 2) both SMFP and NaF (Signal, family protection 1450 ppm). In total 6 expectorated saliva, toothpaste and rinse were collected (3 subjects × 2 types of toothpaste). The samples were made up to a constant volume of 50 ml using diH_20 and vortex stirred for 5 minutes as suggested previously.
Each stirred expectorated saliva/toothpaste and rinse sample was then divided into five 10 ml aliquots. Each aliquot was treated in triplicates by either:

1. diffusing overnight using the HMDS acid-diffusion method (n=9),
2. centrifuging for 10 minutes at 930g at room temperature (n=9),
3. filtering using a syringe filter of 0.2-μm pore size (n=9),

4. centrifuging and then filtering (n=9),
5. no pre-treatment (n=9).

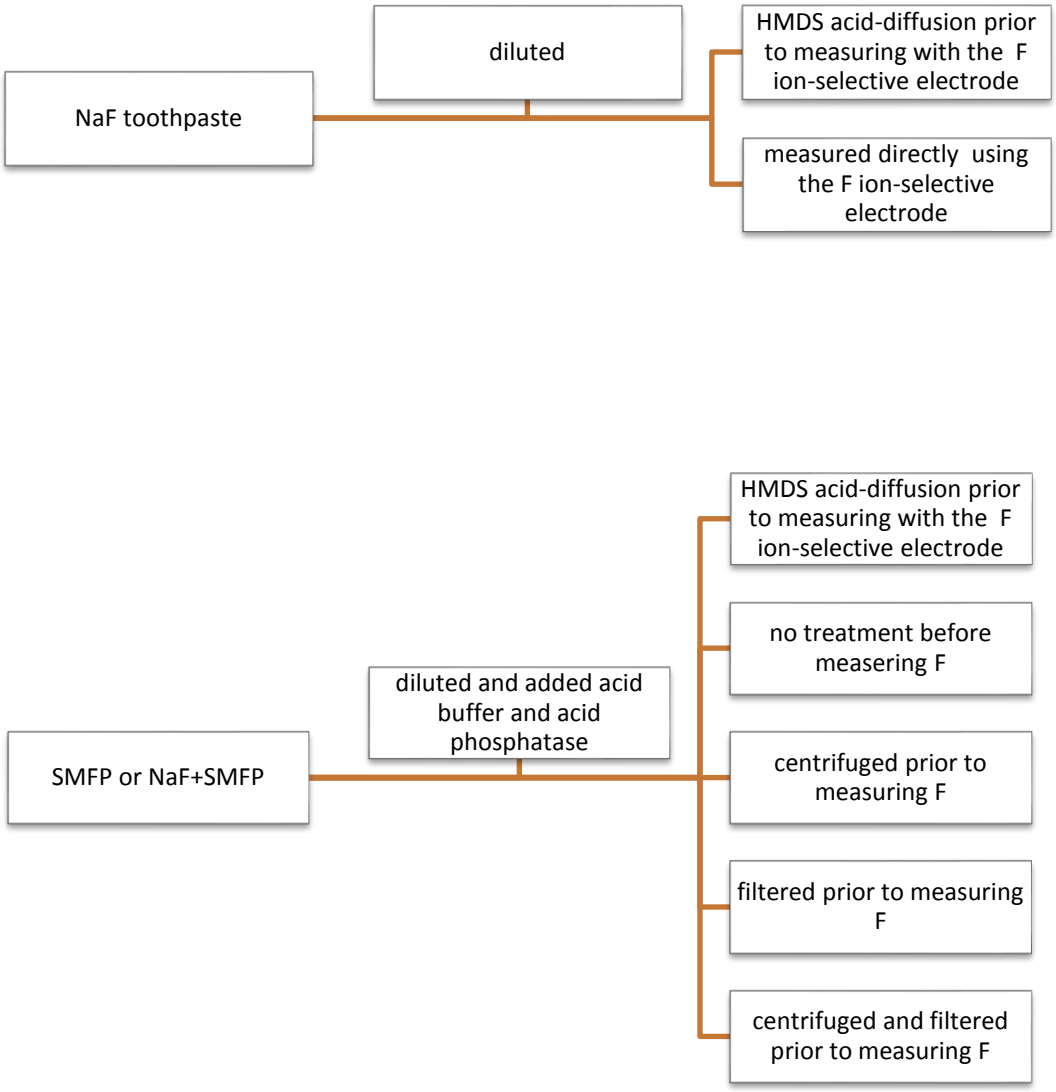
The fluoride concentration of aliquots 1 was measured by F ion-selective electrode after over-night HMDS acid-diffusion (Taves 1968, Venkateswarlu & Vogel 1996, Soto-Rojas *et al.*, 2005).

The fluoride concentration of aliquots 2 to 5 which contained fluoride only as NaF (Aquafresh) were measured directly in triplicate, using the F ion-selective electrode after adding TISAB III (Venkateswarlu & Vogel 1996, Martinez-Mier *et al.*, 2004). Aliquots 2 to 5 which contained fluoride as both NaF and SMFP (Signal) were added 0.1 ml of 5 units /ml acid phosphatase (P1146-50UN-sigma-Aldrich) and 0.1 ml acid buffer (pH=4.8) and then incubated at 37°C for three hours. The fluoride concentration of the samples were then measured using the F ion-selective electrode after adding TISAB III (Duckworth *et al.*, 1991).

The amount of fluoride in dispensed toothpaste (g) was calculated by multiplying the weight of dispensed toothpaste (g) by the fluoride concentration of toothpaste (µg/g). The nominal fluoride concentration (µg/g) of each of the expectorated saliva samples was then obtained by dividing the amount of fluoride in dispensed toothpaste (µg) by the total volume of the rinse and expectorated saliva (ml). The amount of fluoride swallowed was calculated by subtracting the amount of fluoride in the expectorated saliva from the amount of fluoride in dispensed toothpaste. The nominal values were then compared with the corresponding fluoride concentration measurement of the expectorated saliva/toothpaste. Analysis Of Variance (one way ANOVA) was conducted to compare the results obtained from different pre-treatment conducted on samples prior to measuring their fluoride concentration with the F ion-selective electrode. The results of measured fluoride concentration using HMDS acid-diffusion method was then compared with the treated and untreated samples.

A summary of the procedures and analysis used for both toothpastes and expectorated saliva toothpaste and rinse is presented in Figure 4.1.

Figure 4.1 Summary of the procedures involved in measuring fluoride concentration of toothpaste and expectorated saliva, toothpaste and rinse after brushing



4.4. Results

- **Toothpaste**

Results of the fluoride concentration of toothpaste samples treated and measured differently are presented in Table 4.1. The fluoride concentration of toothpastes measured by HMDS acid-diffusion was 10% and 22% higher than the labelled values for NaF and SMFP toothpastes respectively. When fluoride concentration of the samples was measured directly by F ion-selective electrode after adding TISAB III, the results for toothpastes contained NaF were 2% lower than the labelled values while the corresponding values were substantially lower (90%) for samples contained fluoride in the form of SMFP and combination of both NaF and SMFP. By treating toothpaste samples with enzyme (0.1 ml), the measured fluoride concentration were found to be 11% and 5% lower than the labelled values for samples containing fluoride as SMFP and combination of both NaF and SMFP respectively. No significant difference was observed between the fluoride concentration of samples added 0.1 and those added 0.2 ml enzyme.

- **Expectorated saliva, toothpaste and rinse**

This study was a pilot aimed to investigate the most suitable method that can be used for the analysis of toothpastes and expectorated saliva. Sample size was not calculated in this pilot as the main consideration was the number of the analytes prepared for the analysis. For each subject the number of analytes collected for each of the five treatment/analysis was 3. In total 18 samples were collected from all 3 subjects for each analysis/treatment which made the final number of the samples to 90.

The nominal and measured fluoride concentration of expectorated saliva, toothpaste and rinse as well as the amount of fluoride swallowed per brushing is presented in Table 4.2.

The mean (SD) measured fluoride concentration of the samples which were acid diffused for all subjects and types of toothpaste was 9.80 (2.40) µg/ml. While the mean (SD) measured fluoride concentration of expectorated saliva for samples which were treated and/or untreated prior to measuring fluoride with F ion-selective electrode was 5.34 (1.8) µg/ml for all subjects and types of toothpsates.

There was no statistically significant difference ($p>0.05$) in the measured fluoride concentration between samples that were treated differently prior to measurement with the F ion-selective electrode. However, the measured fluoride concentration of samples was significantly higher ($p<0.05$) when they were acid diffused. The amount of fluoride swallowed was also found to be negative when the diffusion method was used (Table 4.2).

Table 4.1 Comparison of the labelled and measured F concentration of three types of toothpastes

TP	Type of Toothpaste	Labelled F concentration (ppm)	Measured F concentration			
			Difussion method	Direct M1 (no enzyme)	Direct M2 (0.1ml enzyme)	Direct M3 (0.2ml enzyme)
Aquafresh-little teeth	NaF	1400	1536	1367	1306	1310
Superdrug Junior	SMFP	1000	1221	93	890	855
Signal	NaF & SMFP	1450	1600	149	1380	1360

Table 4.2 Fluoride concentration of expectorated saliva and the amount of fluoride swallowed calculated from each treatment

Subject ID (No of samples)	Toothpaste Brand	Labelled F con (ppm)	Weight of toothpaste (g)	F type	Nominal F con in expectorated saliva (µg)	Measured F concentration in expectorated saliva (µg/ml)					Estimated amount of F swallowed (mg)				
						Diffusion method	Direct method				Diffusion method	Direct method			
							Treatment					Treatment			
							T1	T2	T3	T4		T1	T2	T3	T4
1 (3)	Aquafresh Big Teeth	1400	0.52	NaF	9.77	11.09	6.58	6.40	6.42	6.03	-0.09	0.23	0.25	0.25	0.28
2 (3)	Aquafresh Big Teeth	1400	0.47	NaF	8.83	10.75	6.90	7.42	7.44	6.75	-0.14	0.14	0.10	0.10	0.15
3 (3)	Aquafresh Big Teeth	1400	0.44	NaF	8.26	12.99	7.30	7.27	7.30	7.38	-0.35	0.07	0.07	0.07	0.07
1 (3)	Signal, family protection	1450	0.33	SMFP + NaF	6.27	8.96	4.02	4.06	4.20	3.80	-0.20	0.17	0.17	0.16	0.19
2 (3)	Signal, family protection	1450	0.37	SMFP + NaF	7.20	8.84	4.20	4.27	4.25	3.92	-0.12	0.22	0.22	0.22	0.24
3 (3)	Signal, family protection	1450	0.33	SMFP + NaF	6.42	6.08	2.99	3.10	3.05	2.95	0.02	0.25	0.25	0.25	0.26
Mean	-	-	0.41	-	7.80	9.79	5.34	5.42	5.45	5.14	0.15	0.18	0.18	0.18	0.19
SD	-	-	0.01	-	1.40	2.40	1.81	1.85	1.85	1.82	0.13	0.07	0.07	0.08	0.08

T1: Centrifugation, T2: Filtration, T3: Centrifugation and filtration, T4: No preparation

4.5. Discussion

4.5.1. Toothpastes

The results showed that fluoride concentration of toothpaste samples containing fluoride in the form of NaF only can be acceptably measured directly with the F ion-selective electrode after adding TISAB III to the diluted samples. However, for the toothpaste samples containing fluoride in the form of SMFP, the fluoride should be released first in order to be detectable by the F ion-selective electrode. In this study toothpaste samples containing SMFP or NaF and SMFP were hydrolysed to fluoride and orthophosphate by adding phosphatase enzyme and incubating the samples for at least 3 hours and also by overnight HMDS acid-diffusion method. According to the results, the HMDS acid-diffusion tended to overestimate the fluoride concentration of toothpastes when compared to the labelled concentration. Whereas measured fluoride concentration of the toothpaste samples treated by the addition of phosphatase enzyme, showed lower values than labelled values. The measured fluoride concentration in a tube of toothpaste may differ from the amount stated on the manufacturer's label for various reasons. Changes in free ionic fluoride as well as stability of the soluble fluoride in toothpastes, during storage may contribute to this difference. Both variables could be decreased during the storage (Freitas 1984, de Oliveira Conde *et al.*, 2003). However, the highest loss up to 40% was reported to occur at room temperature (de Oliveira Conde *et al.,* 2003). In a report by The US Food and Drug Administration (FDA, 1980) it has been stated that the soluble fluoride ions in both types of toothpastes (NaF and SMFP) should not be less than 60% of the total fluoride content. Therefore, the results of this study of which the measured values were within 89% of the labelled values are acceptable. In a study comparing fluoride ingestion from toothpaste in 1.5 to 3.5 year old children from seven European countries (Cochran *et al.,* 2004b), 188 tubes of toothpastes with different batch numbers were analysed using F ion-selective electrode after filtration through 0.2-µm filter. The study also treated toothpastes contained SMFP by phosphatase enzyme. Results showed that 25% of the measured values agreed with the labelled values compared with 59% and 16% lower and higher

than the labelled values respectively. In the present study all the measured values by direct method were lower than the labelled values.

4.5.2. Expectorated saliva, toothpaste and rinse

In order to establish the best method for fluoride analysis of samples of expectorated saliva, toothpaste and rinse, the samples were collected and analysed for their fluoride concentration using the F ion-selective electrode. To remove food and other fine particulates as well as any interfering ions or extraneous material, centrifugation and filtration with 0.2 µm pore filter has been suggested (Van Loveren *et al.*, 2004). However, the results of this study showed no significant differences in the fluoride concentration of samples that were either "centrifuged" or "filtered" or both "centrifuged and filtered" with those that did not receive centrifugation or filtration. In this study the volunteers had their breakfast before attempting the experiment. Since sample collection was conducted almost 2 hours after breakfast no food particulates would have remained in their expectorated saliva. Thus no pre-treatments would have been required for studies with similar designs. However, the studies of this nature should check the time of last food consumption in order to determine the type of treatment.

The results showed the tendency of HMDS acid-diffusion to overestimate fluoride concentration which in turn led to underestimation of the amount of fluoride swallowed. It is therefore recommended to analyse expectorated saliva, toothpaste and rinse as well as toothpastes when estimating fluoride intake from toothpaste ingestion by the same method and not rely on the fluoride concentration of labelled toothpaste.

4.6. Conclusion

According to the results of this chapter it can be concluded that:

1. Fluoride concentration of samples of toothpaste slurries as well as expectorated saliva, toothpaste and rinse containing fluoride in the form of NaF can be measured directly with the F ion-selective electrode after dilution and adding TISAB. While the samples containing SMFP or

combination of NaF and SMFP should be hydrolysed by acid phosphatase prior to measuring with the F ion-selective electrode.

2. Centrifugation and filtration of expectorated saliva, toothpaste and rinse are recommended if the volunteers consumed food since their last tooth brushing.
3. Adding 0.1 ml enzyme to the samples at pre-treatment stage is usually adequate to release fluoride ions from PO_3F^{2-}.
4. When estimating fluoride intake of individuals from toothpaste ingestion, fluoride concentration of toothpaste should be measured as well as the fluoride concentration of expectorated saliva, toothpaste and rinse by the same method.

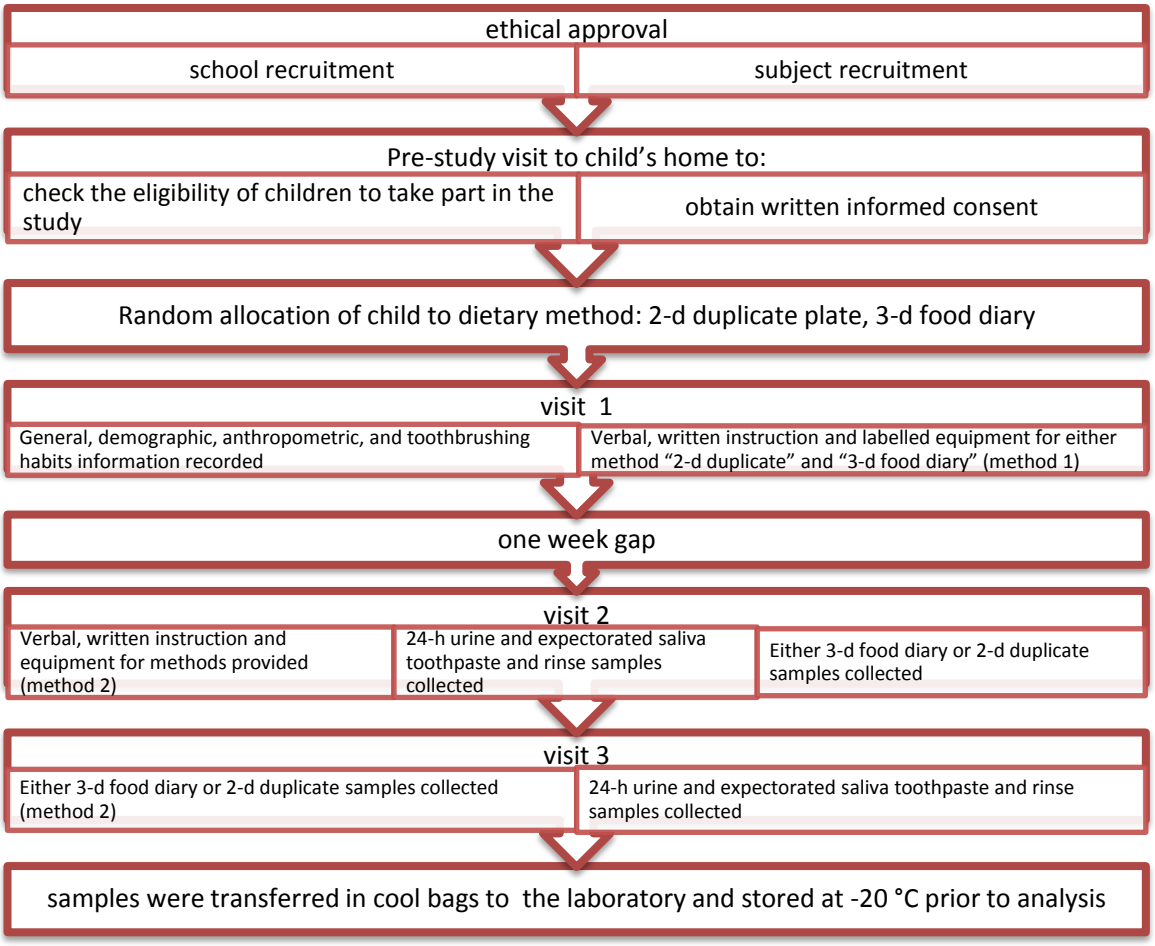
Chapter 5 General materials and methods

5.1. Introduction

In this chapter methods of sampling, the choice of the schools and the subjects are described. In addition, methods of data collection including general (such as anthropometric and demographic data), dietary data, 24-h urines and expectorated saliva, toothpaste and rinse are presented.

Summary of the data collection procedure is presented in Figure 5.1.

Figure 5.1 Flow diagrams for procedures of data collection (field work)



5.2. Materials and Methods

5.2. 1. Ethical considerations and approval

Approval for the study was obtained from the School of Health and Social Care Ethics Committee, Teesside University (Appendix 1) followed by approval from a National Research Ethics Committee; County Durham & Tees Valley 1 Research Ethics Committee (Appendix 2).

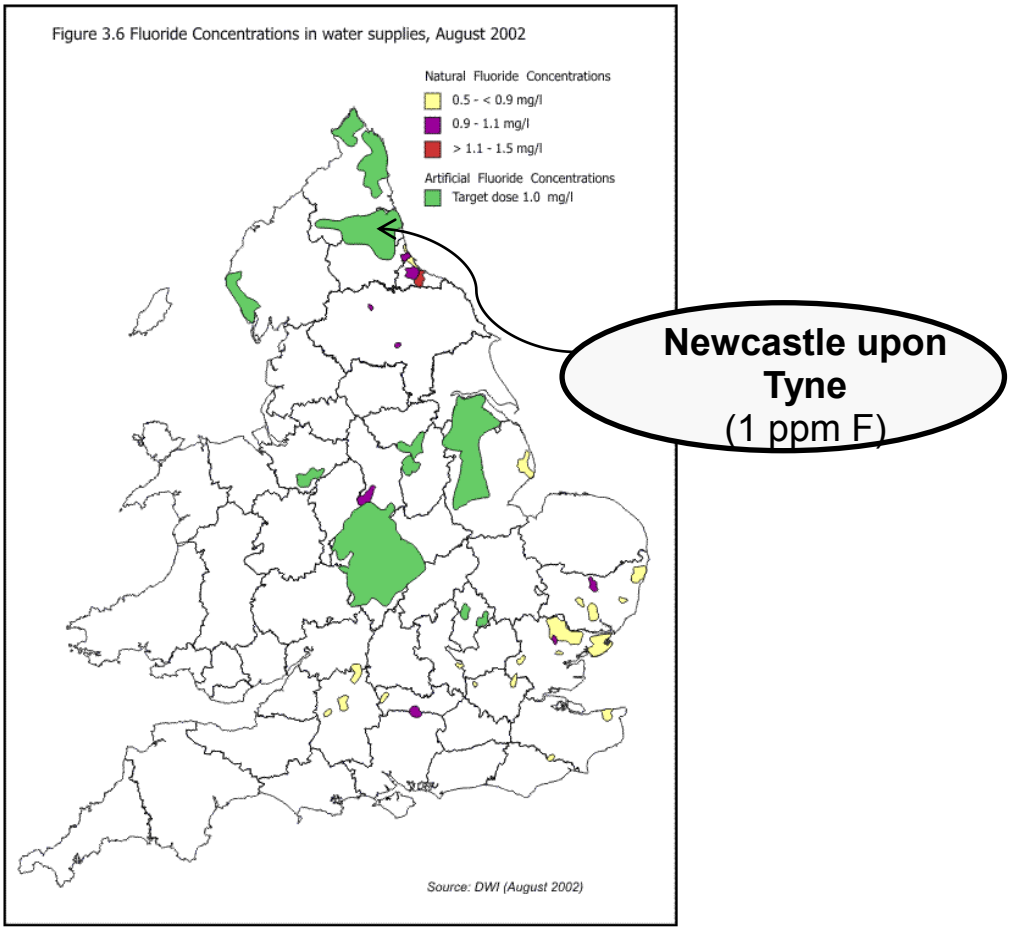
5.2.2. Study location

The study was conducted in Newcastle upon Tyne where the fluoride concentration of water supply is adjusted to 1 ppm (Figure 5.2).

Newcastle is located in the north-east of England with average maximum temperatures of 23°C in July and 3°C in February.

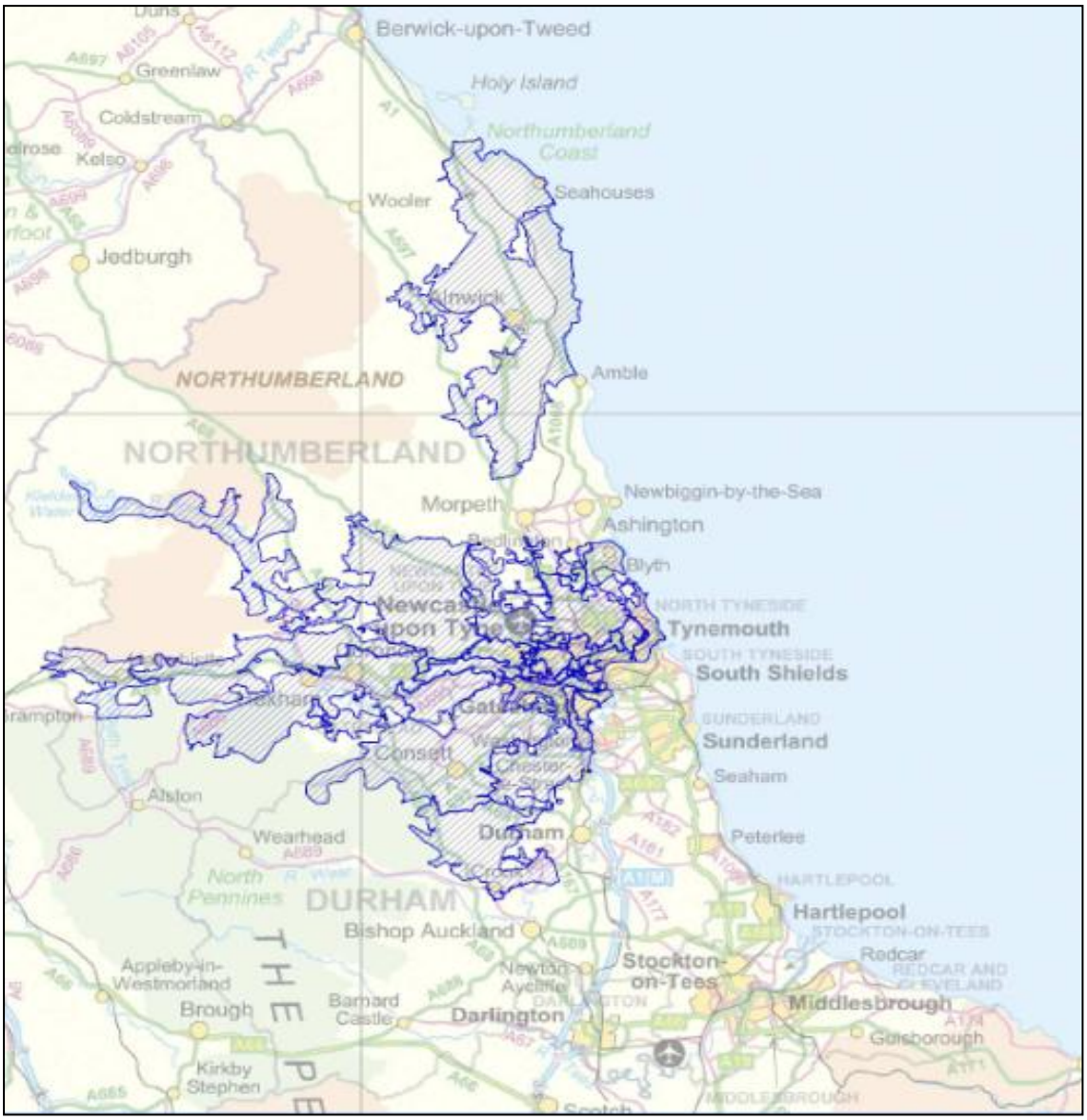
Figure 5.2 Map of fluoridated water areas in i) England, ii) Newcastle upon Tyne

i) England



Source: http://www.bfsweb.org

ii) Newcastle upon Tyne



Source; Northumbrian water (2009)

5.2.3. Subjects and sampling unit

5.2.3.1. Subjects

The subjects were healthy children, chosen from different primary schools for the study.

- **Age**

Children, aged between 4 and 6 years at the start of the study, were eligible for inclusion. Date of birth for each child was obtained from parents.

- **Gender**

In identifying any possible differences in the subjects in relation to the parameters of the study, such as feasibility of urine collection and completeness of different aspects of the study, the sample was designed to include subjects from both genders.

- **Social class**

Since performance and suitability of each dietary method could vary amongst different social classes, subjects were selected from both high and low social areas in order to measure any difference in the completion rate and acceptability of the methods among different socio-economic groups.

- **Sample size**

The sample size was estimated based on the confidence interval, derived from the difference in mean total dietary fluoride intake resulting from the use of the two dietary assessment methods (with a correction for coverage given by an 80% probability that the observed confidence interval is no wider than the specified value). The desired precision of estimation was, therefore, the mean difference ± 1/3 of the standard deviation with a two-sided 95% confidence interval. This resulted in a required sample size of 44 participants which allowed the difference between methods (bias or accuracy) to be estimated to within ± a 1/3 of a standard deviation (a small to moderate effect size). Allowing a 30% attrition rate resulted in a total sample size of 60.

5.2.3.2. Recruitment

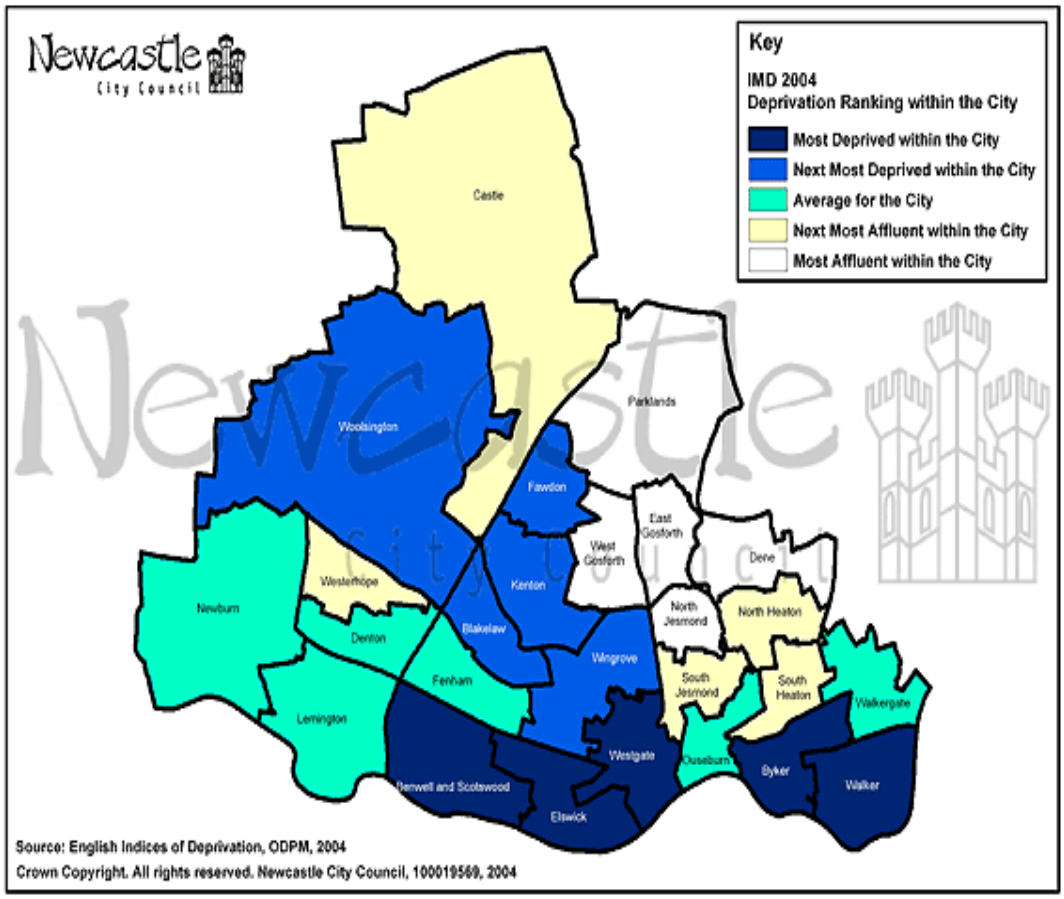
In view of the age range selected for this study, targeting primary schools was considered the best approach for the recruitment of individuals.

- **School recruitment**

According to the Newcastle City Council website, the city is divided into 26 wards. Based on the Index of Multiple Deprivation (IMD) (Department for Communities and Local Government 2008) (Newcastle city council 2007) the wards are aggregated into 5 Bands, with Band 1 being the most deprived, and Band 5 the most affluent wards (Appendix 3). Division of the city into five Bands is illustrated in Fig 5.3.

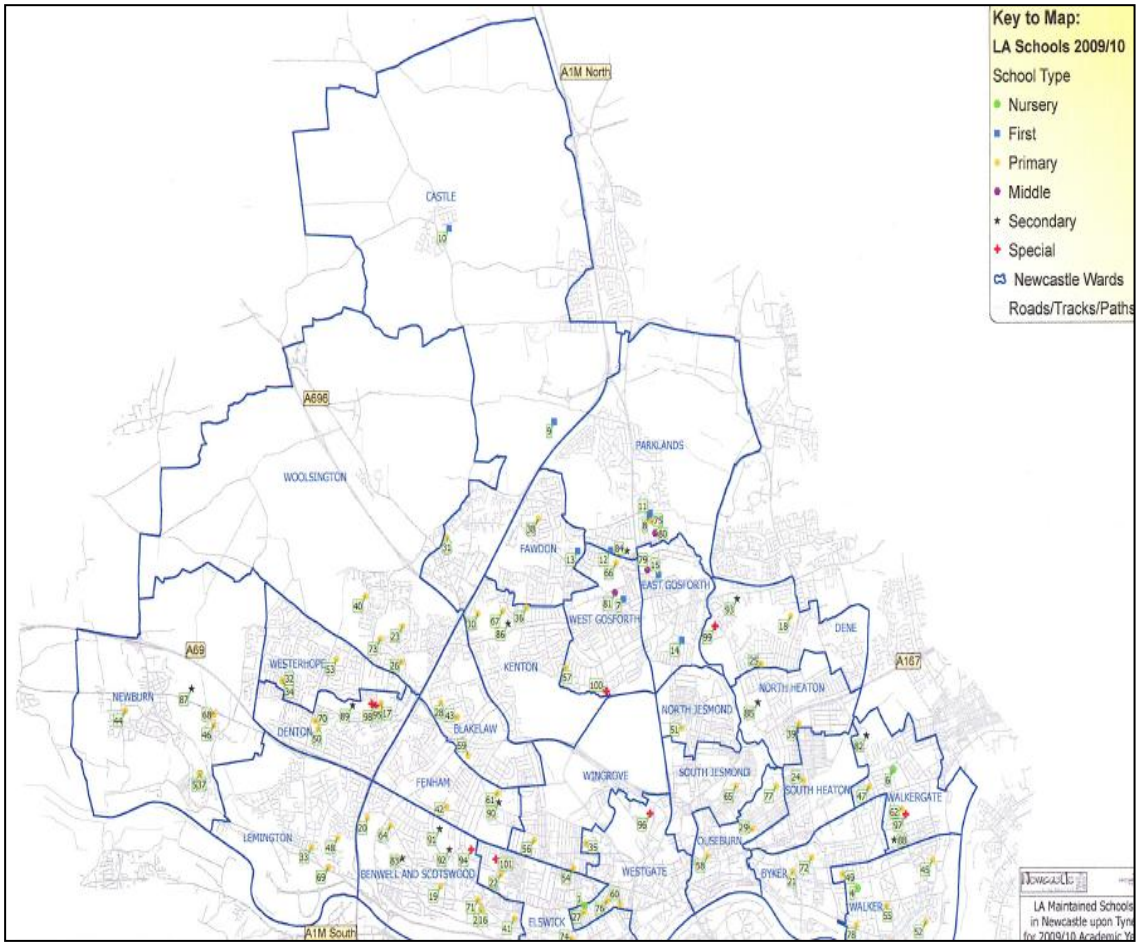
Information on the number of primary schools and their distributions within the five Bands was also obtained from the city council website, and is shown in Figure .5.4.

Figure 5.3 Map of city Bands in Newcastle upon Tyne based on IMD score (2007)



Source Newcastle city council website : (http://www.newcastle.gov.uk/core.nsf/a/imdwardanalysis)

Figure 5.4 Distribution of schools in 5 city Bands in Newcastle



Source:http://www.newcastle.gov.uk/wwwfileroot/educationlibraries/admissions/Key_to_schools_and_map.pdf

There were 70 primary schools in Newcastle (Table.5.1) of which 35 from Bands 1,4 and 5 were selected. The schools were contacted by letter (Appendix4) in April 2009 inviting them to take part in the study. The schools were then followed up by phone for expression of interest, and appointments were arranged with the head teachers of the schools.

At the meetings the head teachers were explained the head teachers and they were given a parents' recruitment pack which comprised: parents' invitation letter (Appendix 5), Study Information Document (Appendix 6), and the response form (Appendix 7). The head teachers were then given time to consider whether their schools to take part in the study.

Table 5.1 Schools recruitment information

Band*	Total No of schools
1	21
2	10
3	20
4	7
5	12
Total	70

*(Newcastle City Council 2007)

- **Subject recruitment**

Recruitment packs were distributed to classes of 4-6 year olds in the schools which agreed to take part in the study, and subsequently parents were invited to attend an introductory session at their child's school. Arrangements were made with the schools to collect the returned response forms from the parents who had expressed an interest to take part. Consequently, the first set of home visits were arranged by the researcher. In the first visit, parents were given an opportunity to ask any questions they had regarding the study and the eligibility of their child in relation to participating in the study. The process of selecting children has been based on the following criteria:

i. Inclusion criteria

- Child between 4-6 year old of either genders;
- Child with no dietary restrictions;
- Child with no chronic and metabolic disease and urinary infection;
- Child with no oral disease (no tooth or gum pain) and no dental treatment such as the use of fluoride gels or filled tooth for at least three months prior to the start of the study;
- Child who had been living in Newcastle area since birth;
- Child available to complete both procedures involved the study.

ii. Exclusion criteria

- Child on medication;
- Child with a restricted diet;
- Child with any health problems including chronic metabolic and renal disease

- Child with any other illnesses including chest infection and temperature

Children who met any one of the above exclusion criteria were excluded from the study. Written informed consent (Appendix 8) was obtained from the parents of eligible children. Finally, appropriate dates were arranged with parents to commence the study.

5.2.4. Data collection

For the purposes of anonymity and confidentiality, each child was assigned an identification number (ID) at the time of recruitment and that the number was used throughout the study. Each family was visited at least three times to obtain the relevant information and samples.

5.2.4.1. Demographic data and anthropometric measurements

Demographic information about each child including name, home address, date of birth, gender and name of the school was recorded (Appendix 9). The height and weight of each child were measured and recorded on the anthropometric data collection sheet during the first visit (Appendix 9). Height was measured using a portable stadiometer (DE56618903; ADE, Germany) vertically without shoes to the nearest 0.5 cm. Weight was measured using a portable digital scale (SOEHNLE, Slim Design Linea, Germany) without shoes and jacket to the nearest 0.1 kg. The same weight and height scales were used throughout the study to avoid any possible measurement errors. In addition, each time that the weight scale was used it was calibrated using a standard 10 kg weight.

5.2.4.2. Allocation of the dietary assessment methods

This study was a randomised cross-over study, comparing observations within individuals. Each child underwent two data and sample collection sessions in which a different dietary method was used, with an interval of approximately one week between each session.

The two dietary methods were coded as follows:

A: 3-day food diary

B: 2-day duplicate collection

Children were randomised sequentially and a randomisation list (Appendix 10) was prepared prior to the recruitment in order to allocate the order of each dietary methods.

5.2.4.3. Dietary data collection

- **3-day food diary**

Dietary data was collected using a 3-day food diary, with a post collection interview on the fourth day. Parents were given a food-diary to complete for 3 days. The food diaries (Appendix 11) were robust and pocket-sized to be carried out easily by parents.

The front page of each diary consisted of the child's ID number, the dates and days on which the intake should be recorded. Instructions on how to record food and drink items were printed on the first page and an example of how the items should be recorded on the second page of the diary.

Parents and their children were also instructed verbally on how to record details of consumed items over three consecutive days, days: 1-3 (Zohouri *et al.*, 2006).

Parents were asked to use household measures such as spoons and cups to estimate the amount of food and drinks consumed and provide the recipe(s) for home-made dishes at the end of food diaries using the same measures. In addition, parents were given pre-labelled polystyrene containers and tubes and asked to keep a sample of approximately 5g of any homemade food or drink consumed by the child in the containers and keep them in the fridge.

Two samples of tap water were collected from each family on the second and third visits. The samples were kept in 7 ml bijous[1].

The purpose of the study was also explained to children using simple, appropriate language to persuade them to co-operate more actively and tell their parents what they ate.

If a child took a packed lunch to school, his/her parents were asked to record everything they put in their child's packed lunch and to ask their child to bring back the leftover. Parents then recorded the leftovers.

If the child was receiving school dinner, parents asked their child to recall what she/he had for lunch. Parents also asked their child to recall if they had swapped any food and drinks at school or drank tap water from the school's fountain.

All schools provided a free midday snack (a portion of fruit or a bottle of milk) for children. Parents asked their child to recall the type and amount of snack they consumed at school and recorded this information in the food diary.

[1] 7 ml bijou by Scientific laboratories, UK

In addition, the researcher contacted the schools directly and recorded the type of midday snack and requested lunch menus for those particular dates, when study children were completing diaries.

On the fourth day a private interview with parents and their children was carried out at the child's home by the researcher, after the 3-day food diary had been completed.

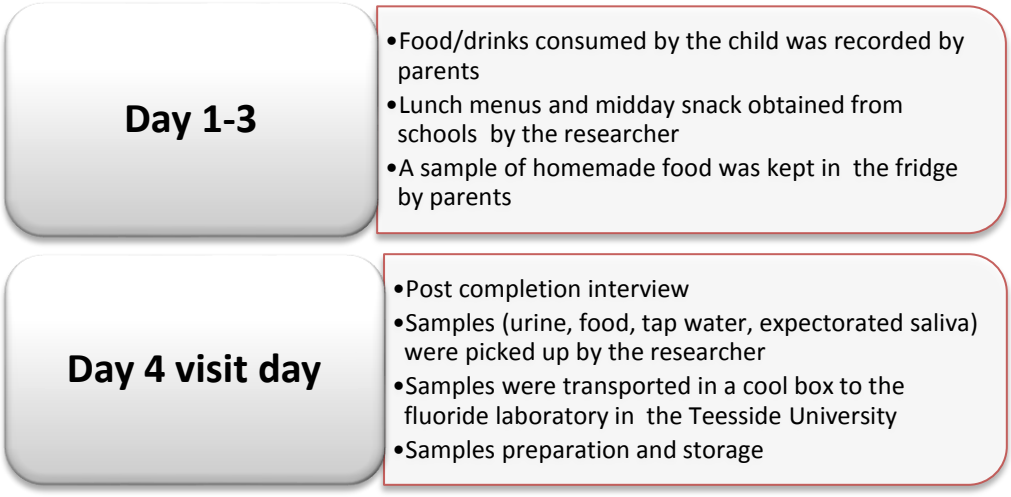
Completed food diaries were examined with the parents and children in order to:

1. Ensure all food and drink items were entered into the diary.
2. Clarify the type of food and drink, e.g. fresh fruit juice or fruit juice from concentrate or squash.
3. Obtain or check the recipe of any homemade dish and drink consumed by child.
4. Estimate the weight of food and drinks consumed by the child using a food portion atlas (Nelson *et al.*, 1997). The food atlas illustrates the size of food and drink items and household measures as a life size picture.

Homemade food and drink samples were kept in a cool box and taken to the Research Laboratory at Teesside University, Middlesbrough on the same day.

A summary of the procedures is demonstrated in Figure 5.5.

Figure 5.5 Summary of data collection procedures by the 3-day food diary method



- **2-day duplicate method**

Parents were provided with written instructions on how to duplicate all food and drinks consumed by their child over two consecutive days, one week day and one weekend day (Guha-Chowdhury *et al.,* 1996, Rojas-Sanchez *et al.,* 1999) (Appendix 12).

In addition, the collection method was explained and demonstrated to the parents by the researcher.

Two sets of containers and bottles labelled with the child's ID number, and the day number (Day 1 and Day 2) were given to parents with a description of what should be put in each container over the 2-day collection period.

Parents were instructed to follow the usual dietary habits and duplicate as precisely as possible everything their child actually ate and drank over two days.

They were also instructed to remove parts of food not normally eaten such as cores, skins, and bones before including the food in the containers.

In the case of cooked meals, they were instructed to serve two similar portions on two separate plates and wait until the child finished her/his portion, then add food to or remove food from the comparable portions.

Parents were instructed to keep all types of solid foods as well as milk for each day (Day 1 and Day 2) in the container labelled for that day. The drinks and waters were duplicated similarly. All types of liquids except water were kept together; water was duplicated separately.

Parents of children who received a packed lunch were required to duplicate their child's packed lunch and ask the child to bring back any leftovers.

If a child received a school dinner, arrangements were made with the schools for the researcher to be at the school during lunch time. A duplicate lunch was purchased that was the same as the child's as well as the midday snack from the school (Fig. 5.6).

Figure 5.6 Duplicate collection procedure from school meals

a)

Duplicate of a child's school lunch was purchased

b)

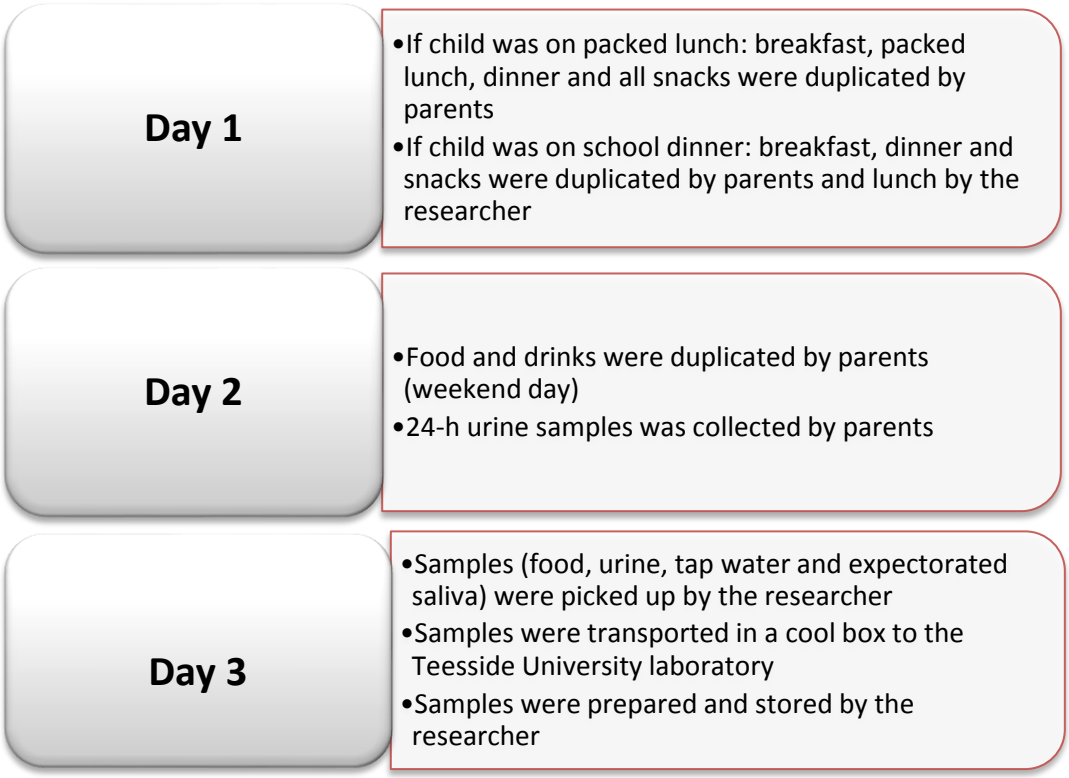
Left: child's plate
Right: duplicate plate

c)

Equal amount of food consumed by child was removed from the duplicate plate and transferred to the containers

Families were visited by the researcher on the day after the completion of the duplicate method (Day 3). Parents were interviewed to ensure that no food or drink item was missed out. The duplicate plate collection buckets were checked for anything with visible stones or parts e.g. bones which would not be eaten.
An overview of data collection procedures has been presented in Figure 5.7.

Figure 5.7 Summary of sample collection procedures by 2-day duplicate method



5.2.4.4. Expectorated saliva, toothpaste and rinse collection

General information about the tooth brushing habits of the children was obtained in the first visit. This captured information on frequency of brushing per day, age of starting tooth brushing, the type of regularly used toothpaste and the person (parent or child) who places the toothpaste on the toothbrush (Appendix 9).
All expectorated saliva, toothpaste and rinse after brushing were collected from each child to obtain data on fluoride intake from toothpaste ingestion.
Children brushed their teeth using their normal toothbrush and toothpaste. The child's toothbrush was weighed before and after dispensing the toothpaste onto

the toothbrush using a portable electronic compact balance (Model HL-100, A&D Instruments, Ltd, UK) in order to record the weight of toothpaste used. Toothpaste was dispensed onto the toothbrush by parent or child depending on the usual habit.

The child and parent were encouraged to use normal practice; brushing with or without assistance and expectorate or rinse depends on their normal practice. However, children were directed to use a cup for rinsing if rinsing after brushing was their normal practice.

The amount of water used for rinsing was also obtained by measuring the weight of water in the rinsing cup before and after rinsing. During tooth brushing and rinsing expectorated saliva mixed toothpaste as well as rinsing water were collected in a pre-weighed labelled pot.

Any toothpaste remaining on the child's face was carefully removed by spatula and added to the pot. Extra water used to rinse the toothbrush was added to the pot. The final weight of the pot was determined to calculate the weight of expectorated saliva. This information was recorded in a toothpaste data recording sheet (Appendix 13) to estimate fluoride ingestion from toothpaste.

Specific information about the toothpaste including brand name, flavour, type of toothpaste (adult or child), form of fluoride (Sodium fluoride or Sodium monofluorophosphate), and fluoride concentration of toothpaste (ppm) from the manufacturer's labelling was also recorded.

5.2.4.5. 24-h urine collection

Written instruction (Appendix14) on how to collect 24-h urine samples was given to parents at the first visit.

Two labelled disposable plastic bottles (1000 ml and 500 ml) with screwed cap, a disposable funnel, several disposable cups and a jug were provided for parents at the first visit by the researcher.

Also, the purpose of 24-h urine collection was explained to parents and the importance of collection of all voided urine over a period of 24-h was stressed to them.

Parents were instructed to collect the 24-h urine sample on the last day of each dietary collection method (Day 3 for the 3-day diary and Day 2 for the duplicate plate method). They were asked to discard the first voided urine in the morning of

the collection day but to record the time and this was marked as the beginning of 24-h urine collection. All urine samples passed by the child after this time were pooled during the 24-h collection period. The first urine passed on the following day (Day 2 of urine collection) was marked as the end of 24-h period. Collected urine samples were then transported in cool bags to the research laboratory by the researcher.

5.3. Sample preparation

5.3.1. Food and drink samples

- **3-day food diary**

a) **Home-made food and drinks**: Each sample of home-made food from the 3-day food diary method was homogenised using a hand blender (Annabel Karmel electric hand blender, distributed by Boots, UK). The sample was then divided into a couple of small zip lock plastic bags, labelled with child ID and food code and stored at -20°C.

b) **Preparation of food/drink items not included in fluoride database**

Fluoride contents (µg/100 g) of 275 food and drink items were previously measured by researchers at Newcastle University, School of Dental Sciences, Department of Child Dental Health. Permission was obtained from Newcastle University to use these data and add them to the nutritional programme: Weighed Intake analysis Software Package (WISP) used to analyse food diaries. In this study 40 more food and drink items consumed by most children but not previously analysed by Newcastle University were identified, purchased and prepared for fluoride analysis in line with the previous study protocol used in Newcastle University. In summary, 10 brands of each item of food or drink were purchased from different supermarkets (supermarket brands and other brands which are available in the UK market). Equal weight/volume of each food or drink was measured and thoroughly mixed to provide a homogenised sample for subsequent fluoride analysis.

The samples were divided into two labelled zip lock plastic bags or bijous and stored at -20°C for fluoride analysis.

- **2-day duplicate**

The duplicates of food, drink, and water consumed were weighed separately using a portable kitchen scale (SALTER, Germany). The information on weight of food,

drink and water for Day one and Day two was recorded separately in a laboratory book.

Duplicate samples were taken to the Newtec laboratories (Billingham, Teesside) where they were weighed out and homogenised by a Thermomix TM31 blender (manufactured by Vorwek, Germany).

Fifty grams of the homogenised food sample for each day (2× per day =4 samples per child) were placed in small zip lock bags labelled with child ID, day number and stored at -20°C. Two 5 ml of duplicate drinks (2× per day=4 samples per child) was also taken into pre-labelled bijous and stored at -20°C.

5.3.2. Urine sample preparation

All the urine samples from the same individual child collected over 24-hour was pooled. The volume of pooled urines was measured and recorded in a urine record information sheet (Appendix 15). The volume of urines was then corrected for 24-h using the following formula:

Corrected urine volume (ml/24-h) $= \frac{\text{Total urine volume (ml)}}{\text{Duration of collection(min)}} \times 1440$, where 1440 is the number of minutes in 24 hour and obtained by multiplying 24 by 60. The duration of urine collection was also calculated in minutes in the same way.

In addition, urine flow rate (ml/h) was calculated by dividing the corrected urine volume (ml) by 24 h.

Six aliquots of 7 ml were taken into bijous. Three of them were labelled for creatinine, nitrogen /potassium, and fluoride analysis while the remaining three were kept as the backup. The labels also contained the child's ID and the method of dietary collection which was conducted along with urine collection (named as method A for 3-day food diary and or B for 2-day duplicate method). All the urines were stored at -20°C for later analysis.

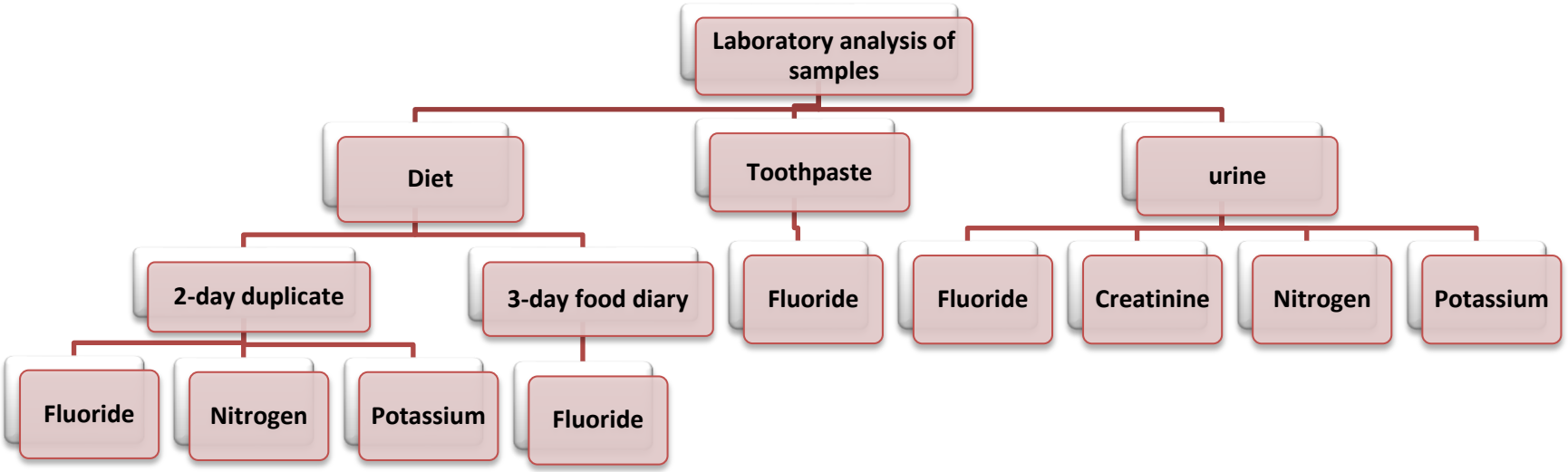
5.3.3. Expectorated saliva, toothpaste and rinse

The collected, expectorated saliva, toothpaste and rinse were immediately taken to the laboratory in cool boxes. Each sample was made up to a constant volume (120ml) before stirring for 5 minutes using a vortex mixer (Chocran *et al.*, 2004b). Three aliquots of the sample were taken into three small bijous (7 ml each), one for analysis and the other two as backup. The bijous were coded with the child's ID, and stored at -20°C.

5.4. Sample analysis

All collected biological and non-biological samples were analysed for fluoride concentration. Duplicates of mixed food and drinks were analysed for nitrogen and potassium content. Urine samples were analysed for creatinine, nitrogen and potassium content. An overview of all the analytical tests carried out on each type of sample is presented in Figure 5.8.

Figure 5.8 Summary of analytical tests carried out on the collected samples



5.4.1. Fluoride analysis

Data and sample collections were completed in November 2009. Once all 24-h urines, expectorated saliva, toothpaste and rinse and food and drink samples were collected fluoride analysis of samples was commenced which was carried out by the researcher in School of Science and Technology, Teesside University and School of Dental Sciences, Newcastle University.

- **Urine samples**

Frozen urine samples were defrosted at room temperature prior to fluoride analysis. Fluoride concentration of the samples was measured by the direct method in triplicate, at room temperature using F ion-selective electrode (Model 9409 Thermo Orion, USA) and meter (Model 720) after adding TISAB III. Prior to measuring fluoride concentration of samples, the electrode was calibrated using a series of standards prepared by adding TISAB III in a proportion of 1:10 (v/v). Concentrations of fluoride standards were chosen to ensure that they covered the range of the expected sample concentrations (Venkateswarlu & Vogel 1996, Martinez-Mier *et al.,* 2011).

- **Expectorated saliva, toothpaste and rinse**

Fluoride concentration of toothpaste samples was measured based on the type of fluoride they contained and as described in Chapter 4, section 4.3.

- **Food and drink samples**

Non-milk based drinks and waters:

Fluoride concentrations of these types of samples were measured directly in triplicate using F ion-selective electrode after adding TISAB III in the proportion of 1:10 (v/v) at room temperature (Martinez-Mier *et al.,* 2011).

Food samples and milk-based drinks:

The HMDS acid- diffusion technique was used to release fluoride ions of the food samples as well as the standards overnight (Whitford 1996, Taves 1968, Venkateswarlu & Vogel 1996, Soto-Rojas *et al.,* 2005). The fluoride concentration of samples was measured in triplicate, at room temperature after adding TISAB III and as described above.

5.4.2. Creatinine, nitrogen and potassium analysis of urine samples

The tests were carried out by James Cook University Hospital (JCUH) in Middlesbrough in December 2009.

- Creatinine concentrations (µmol/l) of urine samples were measured to assess the completeness of 24-h urine collections. The Jaffe method (Taussky 1954) using the auto analyser, ADVIA 1650 (Siemense Medical Solutions Diagnostics) was employed.
- Urea concentration (mmol/l) of each urine sample was measured using the auto analyser ADVIA 1650 (Siemens Medical Solutions Diagnostics), based on the Roch-Ramel enzymatic reaction using urease and glutamate dehydrogenase.
- Potassium concentration (mmol/l) of each urine sample was measured using the auto analyzer, ADVIA 1650, by the K-Ion-Selective Electrode (K-ISE) (Siemense Medical Solutions Diagnostics).

5.4.3. Nitrogen and potassium analysis of food and drinks

All the analyses were conducted in December 2009 by Newtec, laboratories Ltd in Billingham.

- Nitrogen content of duplicate food and drink samples was measured by Kjeldal method on a Grehardt Vapodest 50 protein analyser (Germany), using a Gerhardt Turbotherm digest block kit (distributed by VWR).
- Potassium content of food and drinks was measured with a flame photometer (Jenway PFP 7) after ashing the samples at 525°C ±25°C using Carbolite CSF 1100 Furnace for a minimum of two hours (after charring on a hotplate).

5.5. Validity and reliability of fluoride analysis method

Ten percent of all the samples including food, drink, water, urine, and expectorated saliva, tooth brushing and rinse were chosen randomly and re-analysed to determine the reliability of the employed methods.

5.6. Disposal of the samples

Urine samples were disposed in an allocated toilet. Bijous, containers and bottles containing biological samples (urine and expectorated saliva) were placed in

Virkon solution (1%) (Anachem, UK) for two hours prior to disposal. The containers were rinsed and placed in yellow bags, labelled as clinical waste and disposed of according to Teesside and Newcastle Universities' disposal systems.
The samples of water and drinks were disposed of in the sink while the food samples were placed in black plastic bags and disposed through the rubbish disposal system of the Universities.

5.7. Data Generation

Two types of data: intake and excretion were generated in this study. Intake data were generated from dietary data collected by each method, expectorated saliva, toothpaste and rinse while excretion data generated from 24-h urine collections.

5.7.1. Dietary data

- **2-day duplicate method**

Total fluoride intake (mg/day) = F concentration of duplicate food (mg/g) × weight of food (g/d)
Total nitrogen intake (g/day) = Nitrogen concentration of duplicate food (g/g)× weight of food (g/d)
Total potassium intake (g/day) = Potassium concentration of duplicate food (g/g) × weight of food (g/d)

- **3-day food diary method**

Information recorded in the food diaries was used to estimate total daily energy intake as well as total daily intakes of other nutrients including fluoride, nitrogen, and potassium.
The computerised UK food composition tables (McCance & Widdowson's, 6th edition) (McCance *et al.,* 1991) were used to code food diaries. When there was no code for recorded food such as homemade meals, more than one food code was used: e.g. pasta with bacon would have three separate codes for pasta, bacon, and tomato sauce according to the recipe provided by the parents.
Weighed Intake analysis Software Package (WISP), which is a nutritional analysis programme (Tinuviel, UK) based on nutrient file compiled from McCance & Widdowson's food composition table (5th and 6th edition and its supplements) (McCance *et al.,* 1991), was used to facilitate nutrient analysis.

The program was developed by Tinuviel (Tinuviel Software) for estimating total daily nutrient and energy intake. WISP is designed to allow searching for food codes during data entry and to screen for any unusual data, for example unlikely food codes and food weights. The programme also provides food weight for small, medium, and large portions depending on what has been recorded in the diaries.

The most important nutrient for this study was fluoride. However, WISP as with any other nutrient analysis database, does not include fluoride contents of food, and drink items. Nevertheless, the program is designed to allow the entry of up to 13 new nutrients.

Finally the contribution of each food group to total daily fluoride intake was determined.

5.7.2 Tooth brushing data

Toothpaste ingestion was measured as follow:

i. Fluoride content of dispensed toothpaste was calculated by multiplying fluoride concentration of toothpaste by the weight of toothpaste used.
ii. The amount of fluoride in expectorated saliva was calculated by multiplying concentration of fluoride in expectorated saliva (µg/ml) by the total weight of (g) expectorated saliva, toothpaste and rinse.
iii. The amount of fluoride ingested (mg/d) was then obtained by subtracting the amount of fluoride in expectorated saliva (mg/kg) from the amount of fluoride used from both water and toothpaste (mg).

5.7.3. Excretion data

a) Urinary fluoride excretion (µg/d) =Fluoride concentration (µg) of urine × corrected 24-h urine volume (ml).
b) Total daily creatinine excretion (mg/d)=creatinine concentration (µmol/l) × corrected 24-h urine volume (l/day)
c) Total daily nitrogen excretion (g/day) =Urea concentration (mmol/l)×24-h urine volume (l/day)[2]
d) Total daily potassium excretion (g/day) = potassium concentration (mmol/l) × corrected 24-h urine volume (l/day)[3]

[2] Conversion factor of 28.01

e) Fractional Urinary Fluoride Excretion: (FUFE(%)=[Urinary fluoride excretion (mg/d) /total fluoride intake (mg/d)]×100
f) Total daily fluoride retention=Total fluoride intake (mg/d) – total daily fluoride excretion (mg/d) (including 10% losses of intake through faeces)
g) Fractional fluoride retention (%)=[Total daily fluoride retention (mg/d)/total daily fluoride intake (mg/d)] ×100

5.8. Data analysis

All the generated data were entered into several Microsoft excel datasheets, once completed; a summary file containing the main variables was generated.

Descriptive analysis were derived using statistical package SPSS (version 17.0).

Paired t-test was conducted to compare dietary data at the group level. While dietary data at individual level were compared by Bland Altman method by which the difference in scores of two measurements was plotted against the mean for each subject.

Regression analysis was also conducted to examine the association between total daily fluoride intake and:

- Daily urinary fluoride excretion
- Fractional urinary fluoride excretion
- Total daily fluoride retention
- Fractional fluoride retention

The typical within-child error in dietary F intake from one measurement (day) to another was calculated by dividing SD of differences by √2. Where there were substantial relationships between the difference and the mean, data were first log transformed providing ratio limits of agreement (Bland & Altman 1999).

5.9. Outcome variables

The outcome variables were generated for the present study, are listed in Table 5.2.

[3] Conversion factor of 39.1

Table 5.2 List of general, intake and excretion data generated in the present study

General and demographic data	• Degree of deprivation (band) • Age • Gender • Individual F concentration of supply water (µg/ml)
Anthropometric data:	• Weight (kg) • Height (cm) • Body Mass Index (kg/m^2)
Dietary data	• Dietary method (3-day food diary or 2-day duplicate) • 3-day food diary (day 1, 2, 3) a) Average daily energy intake (kcal/d) b) Average daily potassium intake (g/d) c) Average daily nitrogen intake (g/d) d) Average daily fluoride intake (mg/d) e) Food and drink groups f) Average fluoride intake from each food and drink group (mg/d) • 2-day duplicate collection (day 1,2) a) Weight of collected duplicate food and drink (g) b) fluoride concentration of duplicate food and drinks (mg) c) Average daily nitrogen intake (g/d) d) Average daily potassium intake (g/d) e) Average daily fluoride intake (mg/d)
Tooth brushing habits data	• Frequency of brushing/day • Type of used toothpaste and fluoride content (ppm) of toothpaste • Form of fluoride • Age of starting to brush
Data on fluoride ingestion during tooth brushing	• Weight of dispensed toothpaste (g) • Amount of fluoride in dispensed toothpaste (mg) • Amount of fluoride in expectorated saliva, toothpaste and rinse (mg) • Amount of fluoride ingested from toothpaste (mg/brushing) • Amount of fluoride ingested from toothpaste (mg/d)

Continued Table 5.2.

24-h urine data	• Urine volume (ml/d) • Corrected urine volume for 24-h (ml/24-h • Fluoride concentration (mg) • Urinary fluoride excretion mg/d and mg/kg bw/d • Daily creatinine excretion (mg/d) • Potassium concentration (mmol/l) • Daily potassium excretion (g/d) • Nitrogen concentration (mmol/l) • Daily nitrogen excretion (g/d)
Validation of dietary data	• 3-day method a) Energy intake (kcal/d) b) Physical activity level c) Urine nitrogen/diet nitrogen (%) d) Urine potassium/diet potassium (%) • 2-day duplicate method a) Urine nitrogen/diet nitrogen (%) b) Urine potassium/diet potassium (%)
Assessment of completeness of 24-h urine	• Urine volume (ml) • Urine flow rate (ml/24h) • Creatinine excretion (mg/d)

Chapter 6 Results of the recruitment and general information

6.1 Introduction

In this chapter the results of anthropometric, demographic, recruitment and tooth brushing habits has been presented. A discussion on methods in relation to recruitment and the evaluation of oral hygiene habits has also been provided in this chapter.

6.2. Aims

The aims of this chapter were to evaluate:

- the response and completion rates
- oral hygiene habits

6.3. Methods and materials

Methods of recruitment and general data collections have been presented in Chapter 5, section 5.2.3.

6.4. General results

6.4.1. Recruitment

Recruitment was undertaken in Newcastle upon Tyne between April and November 2009. The recruitment of schools and children was an on-going task to achieve the target number for the study. Data collection was therefore undertaken parallel to recruitment.

- **School recruitment**

In total, 35 schools out of 70 in all bands were contacted, of which 10 (28%) agreed to take part in the study: six from band 1 (a highly deprived area), one from band 4 and three from band 5 (affluent area). The number of schools in each band is presented in Table 6.1.

Table 6.1 School recruitment by band

Band*	Total number of schools	Number of schools contacted	Number of schools agreed to take part in the study
1	21	11	6
2	10	6	0
3	20	0	0
4	7	6	1
5	12	12	3
Total	70	35	10

* Bands are classified according to the deprivation status with band 1 as the most deprived, and band 5 as the most affluent area

- **Subject recruitment**

Table 6.2 illustrates the number of children recruited from the three bands. In total, 1095 invitation letters and information packs were sent to the parents of 4-6 years old in the 10 primary schools which agreed to take part. A total of 85 (7.7%) parents showed their interest after receiving the information but 77 (7%) signed the consent forms. Seventy five (6.8%) parents were not interested to take part and returned negative response forms. A further 16 (1.5%) families dropped out. Therefore, the final number of children who started and completed all aspects of the study stood at 61.

Table 6.2 Summary of recruitment from different bands

Total number of:	Band			
	1	4	5	Total
- invitation letters sent out	580	125	390	1095
- returned response forms:				
• positive	55	11	19	85
• negative	25	10	40	75
- written consent forms obtained	48	10	19	77
- children started the study	38	8	15	61
- children completed the study	38	8	15	61

In line with the aim to compare the study variables between children from affluent and deprived areas, schools from both bands 1 and 5 were contacted in the first place.

However, the number of children recruited from band 5 was not sufficient to achieve the approximate balance in the number of children from both social areas. Therefore, schools in band 4 as the next affluent area after band 5 were also contacted. There were only 8 parents in band 4 and 15 parents in band 5 who agreed to take part and completed the study. Consequently, children from bands 4 and 5 were combined as one social group (affluent) for data analysis.

The socio-economic groups for the present study were therefore comprised of children from:

- Low socio-economic areas (LSE) (highly deprived area band 1)
- High socio-economic areas (HSE) (affluent area bands 4 and 5)

The final number of parents who agreed to take part in the study from LSE areas was higher (n=38) than those from HSE areas (n=23).

Information regarding gender, social class, and age of the recruited children is presented in Table 6.3.

- Gender

In total, 56% (n=35) of the 61 children participated in the study were boys. In both areas, the number of boys was more than the number of girls.

- Age

There were almost equal numbers of children in the three study age groups: 20, 4-year olds, 22 5-year olds and 19, 6-year olds.

Table 6.3 Number of children by gender, age and social class

Age (yrs)	**LSE**			**HSE**			**All**
	Boys	**Girls**	**Both genders**	**Boys**	**Girls**	**Both genders**	
4	8	3	11	3	6	9	20
5	9	4	13	3	6	9	22
6	10	4	14	2	3	5	19
All	27	11	38	8	15	23	61

6.4.2. Anthropometric characteristics of children

Anthropometric characteristics of the children are presented in Table 6.4 a to c.

The mean (SD) age, weight, height and BMI of all children were 5.5 (0.9) years, 21.2 (4.1) kg, 113.1 (7.3) cm and 16.4 (1.9) kg/m^2 respectively (Table 6.4.a). Mean weight, height, and BMI of children from HSE and LSE areas were very

close to one another. The anthropometric characteristics of children were also examined by age and gender.

Mean (SD) weight of 4, 5 and 6-year olds were 19.1 (5.0) kg, 21.0 (2.2) kg, and 23.8 (3.7) kg, and those for height were 107 (7.1) cm, 113.5 (3.5) cm and 119.3 (4.7) cm respectively. BMI of all age groups was found to be similar (Table 6.4.b). The results showed equal BMI for boys and girls (16.4, kg/m^2) despite differences in the mean weight and height between boys and girls (Table 6.4.c).

Table 6.4 Anthropometric characteristics of study children by a) social area, b) age group and c) gender

a)

Social area	No of children	Mean (SD)			
		Age [yrs]	Weight [kg]	Height [cm]	BMI [kg/m^2]
LSE	38	5.1 (0.8)	21.4 (4.3)	113.8 (8.1)	16.4 (1.6)
HSE	23	4.8 (0.8)	21.0 (3.8)	112.0 (5.8)	16.5 (2.3)
All children	61	5.5 (0.9)	21.2 (4.1)	113.1 (7.3)	16.4 (1.9)

b)

Age	No of children	Mean (SD)		
		Weight [kg]	Height [cm]	BMI [kg/m^2]
4	20	19.1 (5.0)	107.0 (7.1)	16.6 (2.3)
5	22	21.0 (2.2)	113.5 (3.5)	16.2 (1.4)
6	19	23.8 (3.7)	119.3 (4.7)	16.5 (2.0)

c)

Gender	No of children	Mean (SD)		
		Weight [kg]	Height [cm]	BMI [kg/m^2]
Boys	35	21.6 (4.0)	114.1 (7.0)	16.4 (1.9)
Girls	26	20.6 (4.2)	111.6 (7.5)	16.4 (1.8)

6.4.3. Fluoride concentration of home tap water

In total, 122 tap water samples were collected. Mean (SD) fluoride concentration (µg/ml) of home tap water in each study band is presented in Table 6.5. The mean fluoride concentration of tap water in all bands ranged from 0.97 (µg/ml) in bands 1 and 5 to 1.03 µg/ml in band 4. Examination of standard deviation showed little variation in fluoride concentration of tap water in all bands.

Table 6.5 Mean (SD) fluoride concentration of tap water by band

Band	**No of samples**	**F concentration (µg/ml)**
		Mean (SD)
1	76	0.97 (0.02)
4	16	1.03 (0.03)
5	30	0.97 (0.02)
4&5	46	0.99 (0.02)
Total	122	0.97 (0.02)

6.4.4. Oral hygiene information

Information on tooth brushing habits of the children is summarised in Table 6.6. According to these findings, 60% of parents from HSE areas claimed to have begun brushing their children's teeth/gum before the age of one compared with only 29% of those in LSE areas. The minimum and maximum age of starting brushing was reported as 4 months (HSE areas) and 48 months (LSE areas) respectively. Most parents stated that their children brushed their teeth twice per day (74% from HSE areas and 66% from LSE areas). Among the children 74% used toothpaste labelled by the manufacturer as children toothpaste with a fluoride concentration ranging from 260 to 1450 ppm. The remaining 26% brushed with a toothpaste labelled as adult toothpaste with a fluoride concentration between 1000 to 1450 ppm.

More than half (56%) of the toothpastes used by children contained fluoride in the form of sodium fluoride (NaF), while 39% and 5% contained fluoride in the forms of sodium monoflurophosphate (SMFP) and combination of both NaF and SMFP respectively.With regard to the flavour of the toothpastes, 95% of all used toothpastes had a mint flavour.

Table 6.6 Information on tooth brushing habits, type and fluoride concentration of toothpastes used by children

	% of children by socioeconomic group		
	LSE [n=38]	HSE [n=23]	All [n=61]
Starting age of brushing (month):			
- ≤ 4	0	4	2
- 5-6	21	13	18
- 7-12	8	43	22
- 13-18	13	9	11
- 19-24	29	22	26
- 25-36	26	9	19
- ≥37	3	0	2
Number of brushing/day:			
- Once	34	26	31
- Twice	66	74	69
Type of toothpaste:			
- Adults	24	26	26
- Children	76	74	74
Form of Fluoride in used toothpaste:			
- Sodium Fluoride (NaF)	54	65	56
- Sodium-monofluoro-phosphate (SMFP)	43	26	39
- Mixed (NaF & SMFP)	3	9	5
Fluoride concentration of used toothpastes (ppm):			
- 260-525	29	17	25
- 1000-1100	39	35	36
- 1350-1450	32	48	39

6.5. Discussion

6.5.1. Study location

Water, as a drink by itself or added to other drinks and foods during preparation and cooking, could be one of the main sources of dietary fluoride. Therefore, a fluoridated area was selected for this study to evaluate the ability of the two dietary assessment methods in estimating fluoride intake.

6.5.2. Adequacy of Sample size

The primary outcome variable for this study was the total dietary fluoride intake estimated by each dietary method. The sample size was calculated based on the total dietary fluoride intake of 0.504 (±0.138) and 0.552 (±0.192) mg/day reported for 12 children living in an optimally fluoridated area (1ppm), when dietary data

were assessed by food diary and duplicate plate methods, respectively (Martinez-Mier *et al.,* 2009).

6.5.3. Recruitment

The head teachers' decision as the gate keepers to participate in the research project has been reported to be influential in school staff to facilitate the recruitment as well as parent's decisions to allow their children to take part (Esbensen *et al.,* 2008, Elder *et al.,* 2008). Therefore, direct contact with the head teacher and staff to secure their support and endorsement was essential. In this study each head teacher was sent an invitation letter and study information document. The meetings with head teachers which were arranged to persuade them to take part were found to be effective.

In the present study direct contact with parents was made after receiving their positive responses. They either had the opportunity to attend the school on the date specified in the letter or being contacted by the phone.

Another potent strategy for the recruitment was considering the incentive for school, and families which could have encouraged schools and parents to participate in the research. However, incentives do not have to be extravagant and should be appropriate for the target population. Current study provided financial incentives for both schools and families. Schools were given book tokens to thank them for their support and parents were provided with £55 supermarket vouchers to compensate the cost of duplicating the food.

6.5.4. Response rate and compliance

To date, no study in the area of fluoride intake and excretion has investigated the differences in the performance and completion rate amongst social groups. In the present study, children were recruited from schools located in both LSE and HSE areas of the city. Although the aim of the study was not to seek any information on the response and completion rate, the recruitment strategy allowed observing any likely differences that might occur between the social areas regarding the response and compliance rate.

- **Schools**

Almost equal number of schools was contacted from both HSE and LSE areas. The input and the involvement that was expected from the schools were limited to

providing us with a list of 4-6 year old children and facilitate the distribution of the study information packs. The number of schools agreed to take part in the LSE area was more than twice that in the HSE area. The lack of interest by schools to take part was based on previous experience of poor response from parents.

- **Parents**

More than 60% of children who took part in this study were from LSE areas since higher number of schools from LSE area took part in the study. The overall parental response rate was low in both areas (7%). Therefore the 61 subjects who were recruited might not be the representative sample of the UK population. However, all 61 subjects completed all aspects of the study and the numbers were sufficient to provide statistical power and allow the validation of dietary assessment methods.

In LSE areas the low response rates was attributed by the head teachers to the low literacy rate among the parents, whereas in the HSE areas this was due to the busy working parents.

A higher parental response rate of 25-30% was reported by Rojas-Sanchez (1999) in a study of 16-40 month old children living in two areas of Indiana, USA and San Juan, Puerto Rico.

In the present study, while no attempt was made to recruit equal number of boys and girls, the final number of boys and girls were fairly close. Previous studies of this nature have not reported or investigated differences between boys and girls in terms of compliance.

- **Data and samples**

In the current study, parental co-operation was very high and participated parents agreed to repeat any part of the study if suspected to be incomplete. All of parents who took part completed all aspects of the study and provided all the required data and samples. In a comparative analysis of two dietary methods in the US Martinez-Mier (2009) reported a high rate of 35% incomplete food diary. In another study to compare total fluoride intake of British children living in optimally, sub-optimally and non-fluoridated areas Maguire et al. (2007) reported that all the 33 recruited children completed all aspects of the study.

The good co-operation of parents in this study can mainly be attributed to the good relationship which was built up with the participants during data collection. Parents were provided with a 24h, 7-days contact number, email address and a

generally flexible approach by the researcher. This enabled them to change arranged dates and times and to contact the researcher with regard to any misunderstanding vis-à-vis food collection and urine collection.

6.5.5. Age and anthropometric data

Current evidence suggests that efforts at prevention of enamel fluorosis should include vigorous attention to fluoride exposure during the first 5 years of life when the calcification of the crown of most permanent teeth starts (Fomon *et al.*, 2000).

However, children younger than 4 years old may not be able to fully control their bladder especially at night and therefore might not be able to provide complete 24-h urine samples. Therefore, for methodological reasons and practicality of data collection, the 4-6 year old age group was selected to ensure parental control and child co-operation for the collection of the 24-h urine.

The mean, weight, height and BMI of children included in the present study were in line with the corresponding data reported for 4-6 year old British children in the National Diet and Nutritional Survey (Gregory *et al.,* 2000). However, mean weight and height of children in the present study were slightly lower than the corresponding values of 23.7 kg and 119.8 cm, reported for 6-7 year old British children (Maguire *et al.,* 2007) which could be due to the age difference.

6.5.6. Oral hygiene habits

- **Age of commencing tooth-brushing**

In the current study, 79% of parents reported that their children began brushing their teeth before the age of 2; which was lower than the figure of 94% reported for British children in 1981 (Palmer & Prothero 1981) but higher than the figures of 50%, 56%, 45%, 41% and 1.7% reported for Canadian (Osuji 1988), American (Levy *et al.,* 1995), Indian (Mascarenhas & Burt 1998), Iranian (Zohouri & Rugg-Gunn 2000b) and Japanese children (Arakawa *et al.,* 1995). In general, children in developing countries tend to begin tooth brushing later in life compared to those in developed countries.

It has been reported that children living in a fluoridated community who began brushing their teeth before the age of 2 years were 11 times more likely to develop

fluorosis compared to those who had begun brushing later. On the other hand, for children living in non-fluoridated areas of Norway, early commencement of brushing with a pea sized amount of fluoridated toothpaste was not found to be a significant risk for dental fluorosis but was associated with substantial decrease in caries prevalence (Pendrys *et al.,* 2010).

The present study showed that higher proportion (91%) of children from HSE areas started tooth brushing before the age of 2 compared with 71% from LSE areas. This finding was consistent with the Colombian study (Franco *et al.,* 2005a) which reported a higher proportion (60%-80%) of families from HSE areas to start bushing their children's teeth before the age of one compared with 33%-36 % from LSE areas. However, as with any study, relying on historical information recalled by the respondent can be an issue and the accuracy of such information may be questionable.

- **Brushing Frequency**

On tooth brushing frequency, findings of this study indicated that a higher proportion (74%) of children from HSE areas brushed their teeth twice a day, compared with 66% from LSE areas. On the whole, however, a majority (69%) of children across all three age and social groups were found to brush their teeth twice daily, this being close to that of the 2003 UK Children's Dental Health Survey where 76% of 5 year olds were reported to brush their teeth twice daily (Pendry *et al.,* 2004). However, a lower proportion of children (44%) in India were reported to brush their teeth twice a day (Mascarenhas & Burt 1998). A study conducted in seven European countries (FLINT study), has reported that in Haarlem, Netherlands 64% of children above the age of 3.5 years old to brush twice daily (Cochran *et al.,* 2004a). In FLINT study the highest proportion of brushing twice per day was reported for younger children between 1.5 and 2.5 year olds from Almada/Setubal (Portugal), Knowsley (UK) and Haarlam (Netherland), at 69%, 65%, and 63% respectively. Brushing more than once a day with fluoridated toothpaste during the first two years of life has been reported to contribute to 34% of fluorosis cases in non-fluoridated communities (Pendrys 2000).

In the current study the proportion of children brushing once a day stood at 31%, very close to that of 27% reported for Knowsley (UK) by FLINT study; whilst it was almost half of the proportion found for 4-6 years old Indian children (Mascarenhas & Burt 1998). A trend in the frequency of tooth brushing with respect to age was reported for Indian children in that while only 10% of children younger than 2 years of age brushed their teeth twice daily, this proportion stood at 44% for the children aged 4-6, more than four times higher.

- **Types of toothpaste used**

Over 95% of toothpastes sold in Europe (Arnadottir *et al.,* 2004), the USA (Franzman *et al.,* 2006) and other western countries contain fluoride in the form of either sodium monofluorophosphate or sodium fluoride. The current study showed that more than half of the children (56%) used a fluoridated toothpaste containing fluoride in the form of NaF. Three-quarters of all children brushed with a toothpaste containing more than 1000 µg F /g, of which 39% contained 1350-1450 µg F /g. The number of children in the HSE areas who used fluoridated toothpaste containing ≥ 1000 µg F /g was slightly higher (83%) compared to those in the LSE areas (71%). A study in seven European countries (Cochran *et al.,* 2004a) revealed that between 69% (Cork, Ireland) and 98% (Almada/Setubal, Portugal) of children aged 1.5 to 4.6 years used toothpaste specifically marketed for children.

A recent review suggested that the use of adult toothpaste in very young children, leading to a higher intake of fluoride from toothpaste ingestion, appeared to slightly increase the risk of mild fluorosis in less deprived, low-caries communities but not in socially deprived high-caries populations (Twetman 2009). The authors therefore recommended that socioeconomic status of a community ought to be considered when making any recommendation on the fluoride concentration of toothpaste and the amount of toothpaste dispensed for infants and toddlers.

The present study found that 74% of children used toothpaste manufactured for children which is comparable with 73% reported for children from Knowsley, UK (Cochran *et al.,* 2004b). With regard to the fluoride concentration of toothpaste, the European study reported that between 0% (Cork, Ireland and Knowsley, UK)

and 60% (Haarlem, the Netherlands) of children used toothpaste with a fluoride concentration of < 400 µg/g. The proportion of children using toothpaste with a fluoride concentration ≥1200 µg/g ranged from 0% in Almada/Sebutal (Portugal) to 27% in Oulu (Finland) (Cochran *et al.,* 2004a). In the present study 39% of children used toothpaste with fluoride concentration ≥1200 µg/g.

Fluoridated toothpaste is available in concentrations of 1000-1500 µg F /g for adults and 500-1400 µg F /g for children. Although higher concentrations of fluoride may be available, the WHO (WHO 1994) recommends that a limit of 1500 µg F /g should be maintained. The recommendations for the use of fluoridated toothpaste vary based on age, fluoride concentration of the toothpaste, and the oral health status of the population.

Relevant current guidelines for the use of fluoridated toothpaste by children in the UK (DoH/BASCD 2009) recommend that children up to the age of 3 years old should brush twice a day with a smear of "at least" 1000 µg F /g toothpaste, whereas children aged 3 to 6 years old should use a pea-sized amount (~ 0.25 g) of toothpaste containing 1350 to 1500 µg F /g. However, earlier recommendations were lower of which a small pea sized of <600 µg F /g toothpaste for children younger than 6 years old, who are at a lower risk of developing caries, and a toothpaste with 1000 µg F /g for children who are in the higher risk category was recommended (Holt *et al.,* 1996). The European Academy of Paediatric Dentistry (EAPD 2009) does not recommend fluoridated toothpaste for children under 6 months old. However, it recommends brushing twice daily with a pea-sized amount of toothpaste containing 500 µg F /g and ≥ 1000 µg F /g for children aged 6 months to < 2 years old and 2 to < 6 years old, respectively.

6.6. Conclusion and recommendations

This study has demonstrated similar response rates between socioeconomic groups and a remarkable completion rate of 100%. The present study, therefore, concluded that socio-economic status of the families was of no issue, and that it had no impact on response rate, acceptability and completion rate.

On the basis of oral hygiene information, the present study suggested that the majority of children were found to brush their teeth twice daily with toothpaste marketed for children and containing ≥1000 µg F /g. However, the socio-economic status of the families had an impact on the oral hygiene habits to the

extent that the greater proportion of children from the HSE areas used a toothpaste with a higher fluoride content as well as brushing more frequently (twice daily).

Chapter 7 Validation of analytical methods, dietary assessment methods and the completeness of 24-h urine collections

7.1. Introduction

This chapter describes validation of dietary assessment methods "2-day duplicate" and "3-day food diary". Based on biomarkers of urines: nitrogen and potassium a set of criteria was proposed for the children of the present study on which basis their dietary data was validated. The results of validation are presented and discussed in this chapter.

7.2. Aim

The main aim of this chapter was to examine validity of "2-day duplicate" and "3-day food diary" methods for the assessment of dietary fluoride intake in children.

The subsidiary aims were to:

- Check the validity of fluoride analytical methods and the completeness of 24-h urine samples,

to measure:

- Urinary excretion of nitrogen and potassium
- Dietary intake of nitrogen and potassium
- Urine to dietary potassium and urine to dietary nitrogen ratios
- Creatinine concentration of urines

7.3. Materials and methods

Analytical methods for the measurement of fluoride, nitrogen, potassium and creatinine of different types of samples have been already addressed in Chapter 5, section 5.4.

7.3.1. Quality control of the analytical methods

- **Fluoride**

The reliability of the fluoride analytical methods was examined by re-analysing 10% (n=60) of all samples including food, drink, urine and expectorated saliva for their fluoride concentration.

The validity of the fluoride analytical methods was also checked by adding a known amount of fluoride to 10% of samples (n=60). The fluoride concentration

of the samples with and without adding fluoride was then measured to investigate the recovery of added fluoride.

- **Nitrogen and potassium content of the food samples**

The quality control of the analytical tests for nitrogen and potassium contents of food was checked by Newtec laboratories Ltd. where duplicates of food were analysed for their potassium and nitrogen contents. A reference sample with a known range of 0.158 -0.194% for nitrogen and 1.66-1.88 % for potassium was used in order to carry out the quality control and determine the accuracy and precision of the analytical methods. All samples were analysed in duplicates.

- **Creatinine, Nitrogen and Potassium content of urine samples**

As part of the quality control procedures within James Cook University Hospital, a Biorad Liquicheck Urine Control Level 1 (Urine low) and Level 2 (Urine high) kit was used to check methods and equipment on a daily basis usually ahead of any sample runs.

In addition to the internal quality procedure, James Cook University Hospital also takes part in inter-laboratory blind studies which measures levels of creatinine, nitrogen and potassium at a sample rate of 3 per month.

7.3.2. Completeness of 24-h urines

The completeness of 24-h urines was checked by measuring creatinine excretion and urine flow rates of samples and comparing them with the Tietz (Tietz 1995) and WHO (1999) reference ranges as described in Chapter 2 section 2.3.2.

7.3.3. Validation of dietary assessment methods

The validity of both dietary methods was investigated by measuring dietary and urinary nitrogen and potassium. The ratios of urine nitrogen to dietary nitrogen (UN/DN) as well as urine potassium to dietary potassium (UK/DK) were then calculated.

The dietary methods were considered valid if at least one of the following criteria was met:

a) Both ratios of UN/DN and UK/DK were between 20% and 100%.

Or:

b) If UN/DN ratio was between 20% and 100% and potassium intake was within the range of the National Diet and Nutrition Survey (NDNS) data reported for 4-6 year olds (Gregory *et al.*, 2000) (Table 7.1).

Or:

c) If UK/DK ratio was between 20% and 100% and nitrogen intake was within the range of NDNS data reported for 4-6 year olds (Table 7.1).

Table 7.1 The NDNS values for daily nitrogen (g/d) and potassium (g/d) intake for 4-6 year old British children by gender (Gregory *et al.*, 2000)

Nutrient	Boys		Girls	
	lower 2.5%	**upper 2.5%**	**lower 2.5%**	**upper 2.5%**
Nitrogen intake (g/d)	4.1	12.3	4.2	10.7
Potassium intake (g/d)	1.04	3.02	1.02	2.72

7.3.4. Other criteria for validation of the food diary assessment method

Since the food diary method can provide detailed information on the intake of all nutrients as well as energy, its validity can be checked by comparing Energy Intake (EI) and Physical Activity Level (PAL) of the subjects with those reference values reported for 4-6 year old British children (Gregory *et al.*, 2000). Energy intake for each child was estimated from food diaries using WISP as described in Chapter 9 section 9.3.4. As another criterion for the validation of dietary methods, Physical Activity Level (PAL) was calculated by dividing the mean recorded EI by the calculated Basal Metabolic Rate (BMR) (Bingham 1994). The calculation of BMR was based on the Schofield standard equation (Schofield *et al.*, 1985) as follows:

Male: BMR (MJ) = 0.095 [wt (kg) +2.110]

Female: BMR (MJ) = 0.085 [wt (kg)+2.033]

In this study a PAL cut-off point of 1.28 which has been suggested by Torun (Torun *et al.*, 1996) was selected for validation of dietary data collected by the 3-day food diary method.

7.4. Results

The results of this chapter are presented in three sections: section one presents the results of the quality control of analytical methods and sections two and three present the validity of the completeness of 24-h urines and dietary methods respectively.

7.4.1. Quality control of analytical methods

- **Fluoride analysis**

Table 7.2 shows the mean difference in fluoride concentration of test to re-test analysis for all types of samples which ranged from 0.003 µg/g for food to 0.018 µg/g for water samples. The typical variability from test to re-test ranged from 0.006 µg to 0.022 µg.

Table 7.2 Results of test to re-test fluoride analysis for 10% of the samples

F concentration (µg/g)				
Sample type	**Number of samples**	**Analysis**	**Re-analysis**	**Mean Difference (95% CI)**
Food	12	0.314	0.317	0.003 (-0.011,+0.018)
Drink	12	0.239	0.229	-0.009 (-0.026, +0.006)
Water	12	0.951	0.934	-0.018 (-0.050, +0.013)
Urine	12	0.784	0.798	0.014 (-0.013, +0.042)
Saliva	12	0.589	0.578	-0.011 (-0.024, +0.011)

The mean recovery of fluoride that was added to all samples was 99.3 % with a range from 98.7 % to 100% (Table 7.3).

Table 7.3 Results on recovery of samples after the addition of F standard

Sample type	Number of samples	Mean (SD) F concentration of sample (mg/l or mg/g)	Added F (mg)	Mean (SD)of sample after addition of F (mg/l or mg/g)	% F recovery
Food	12	0.342 (0.09)	0.2	0.542 (0.06)	100
Drink	12	0.238 (0.08)	0.2	0.435 (0.05)	99.3
Urine	12	0.788 (0.06)	0.5	1.280 (0.05)	99.3
Saliva	12	0.614 (0.12)	0.5	1.100 (0.08)	98.7
All samples	60	-	-	-	99.3

- **Nitrogen and potassium analysis of food samples**

The duplicate analysis of samples were within the reference range of 0.158-0.194% for nitrogen and 1.66-1.88% for potassium. In addition, the samples were within 0.02% and 0.07% of each other for nitrogen and potassium respectively which was deemed acceptable.

- **Creatinine, Nitrogen and Potassium analysis of urine samples**

The results for creatinine, nitrogen and potassium in the urine control samples, (as reported by James Cook University Hospital) were found to fall within the upper and lower control limits which were from 5952 to 12838 µmol/L for creatinine, and 29 to 66 mmol/L and 178 to 315 mmol/L for potassium and nitrogen respectively. Therefore, the measurements were considered to be valid. In addition the results of inter-laboratory blind studies were found to be within the normal ranges achieved by other users of the methodology and supported the conclusion that the data were valid.

7.4.2. Validation of urine samples (Completeness of 24-h urine samples)

The completeness of urine samples were checked by both creatinine and urine flow rates.

- **Creatinine**

Overall, mean (SD) creatinine concentrations of 24-h urine samples collected along with the 3-day food diary and 2-day duplicate methods were 12.83 (5.80) mg/kg bw/d and 13.30 (5.85) mg/kgbw/d respectively which were within the reference range of 8-22 mg/kg bw/d (Tietz 1995). Based on individual validation, 24-h urinary creatinine excretion of 10 children in the 3-day food dairy group and

9 children in the 2-day duplicate group did not fit into the reference range (Table 7.4).

- **Urine flow rate**

Mean (SD) urine flow rates of all children was 13 (6) and 20 (7) ml/h for urines collected during the 3-day food diary and 2-day duplicate plate collections, respectively. According to the WHO (Marthaler 1999) a flow rate of less than 5 ml/h for < 6 year olds and less than 9 ml/h for ≥6 year olds are invalid. Urine flow rates of both collections for all children in the present study met this criterion and were therefore confirmed as valid (Table 7.4).

Therefore, all children were included in the study since those children who did not meet Tietz creatinine excretion criteria, fulfilled the WHO flow rate criteria.

Table 7.4 Number of children who did not meet urine validation criteria

Criteria	**No of incomplete urines**	
	3-day food diary	**2-day duplicate**
Creatinine (mg/kgbw/d)	10	9
Flow rate (ml/h)	0	0

7.4.3. Validation of dietary assessment methods

- **3-day food diary method**

According to the results, dietary data of 48 (79%) children were valid on the basis of criteria "a" i.e. both their urine to diet nitrogen (UN/DN) and urine to diet potassium (UK/DK) ratios were between 20% and 100% (Table 7.5). The remaining 13 children had valid dietary data based on either criteria "b" or "c" as described in section 7.3.3. Dietary data of only 3 children were considered over-reported as the UK/DK ratio for all of them (one boy and two girls) was below 20%. Figure 7.1 also illustrates the distribution of UN/DN and UK/DK ratios for all children.

- **2-day duplicate plate method**

According to the results dietary data of 30 (49%) children were valid on the basis of criteria "a" i.e. both their UN/DN and UK/DK ratios were between 20% and

100% (Table 7.6). Dietary data of a further 16 (26%) children met either the criteria “b” or “c”. Therefore, their dietary data were considered valid. While dietary data of 15 children did not meet any of the criteria. Figure 7.2 also shows the distribution of UN/DN and UK/DK ratios for all children.

Table 7.5 Number of children with invalid UN/DN ratio or UK/DK ratio or both when dietary data collected by 3-day food diary

Subject ID Gender	**N intake (g/d)**	**UN/DN ratio (%)**	**K intake (g/d)**	**UK/DK ratio (%)**	**Validation results**
Boys					
12	6.0	64.0	1.4	124.7	✓
15	6.2	87.4	1.3	183.7	✓
17	8.9	91.0	2.0	136.6	✓
29	6.4	69.7	1.9	106.8	✓
2	10.8	17.3	2.0	24.3	✓
19	12.8	26.7	3.1	17.8	over reporter
23	6.5	10.6	2.0	35.2	✓
46	6.9	17.6	1.1	37.8	✓
Girls					✓
33	7.0	76.4	1.7	153.0	✓
7	9.5	33.8	2.7	17.3	✓
10	9.7	5.4	2.5	11.4	over reporter
18	7.0	14.3	2.0	38.0	✓
45	6.61	17.7	1.4	14.5	over reporter

Table 7.6 Number of children with invalid UN/DN or UK/DK ratio or both when dietary data collected by 2-day duplicate

Subject ID Gender	N intake (g/d)	UN/DN ratio (%)	K intake (g/d)	UK/DK ratio (%)	Validation results
Boys					
2	3.8	82.6	0.8	120.8	under reporter
3	3.1	199.0	0.4	251.4	both under reported
4	10.5	56.1	1.7	107.1	✓
5	10.9	23.6	2.4	12.9	✓
8	5.8	190.6	1.0	149.7	both under reported
12	5.97	83.0	1.4	118.8	✓
15	6.1	99.2	1.3	182.6	✓
17	6.5	88.1	1.7	101.4	✓
19	8.8	57.1	1.2	101.2	✓
28	5.5	48.9	0.7	124.4	under reporter
29	6.7	35.6	1.0	130.4	✓
30	5.1	98.9	0.9	169.9	under reporter
32	10.7	58.5	1.8	135.5	✓
34	4.6	40.7	0.7	123.9	under reporter
40	4.7	118.3	0.9	121.5	both under reported
47	7.7	7.2	1.1	34.7	✓
51	2.7	132.9	0.5	287.5	both under reported
53	7.7	62.3	1.0	104.2	✓
54	7.0	46.8	1.0	106.2	✓
59	3.4	50.2	0.6	119.2	under reporter
Girls					
7	3.63	82.2	0.8	136.6	under reporter
20	10	71.9	1.5	103.3	✓
26	7.7	80.3	0.8	220.3	under reporter
27	7.2	51.4	1.2	104.6	✓
31	6.2	54.8	0.7	171.0	under reporter
33	8.2	38.1	1.3	102.8	✓
37	4.3	66.5	0.7	152.2	under reporter
41	5.2	65.6	1.6	129.9	✓
45	5.9	26.3	0.4	102.8	under reporter
52	6.5	52.2	0.8	129.8	under reporter
60	7.6	56.5	1.1	124.0	✓

Figure 7.1 Distribution of individual's UN/DN and UK/DK ratios based on dietary data from the 3-day food diary method

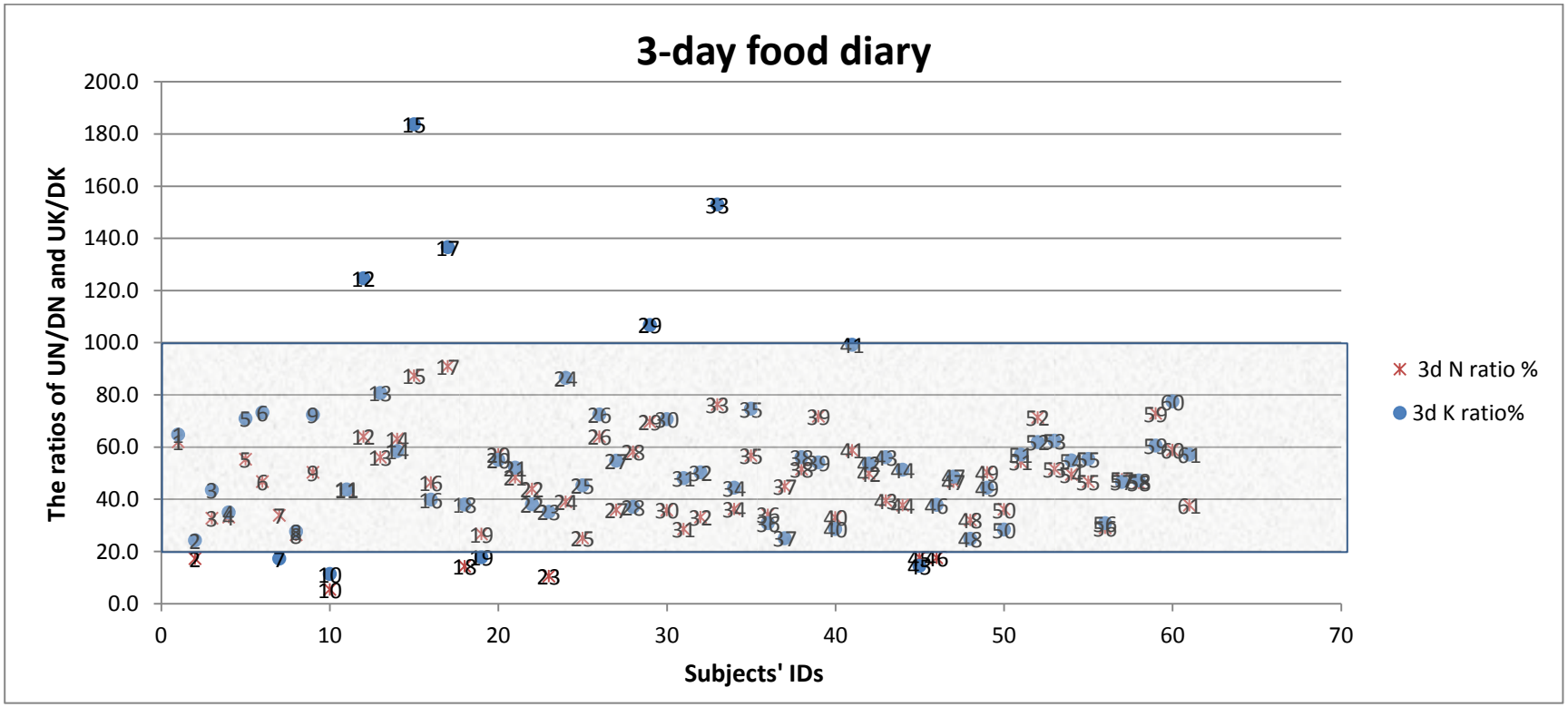
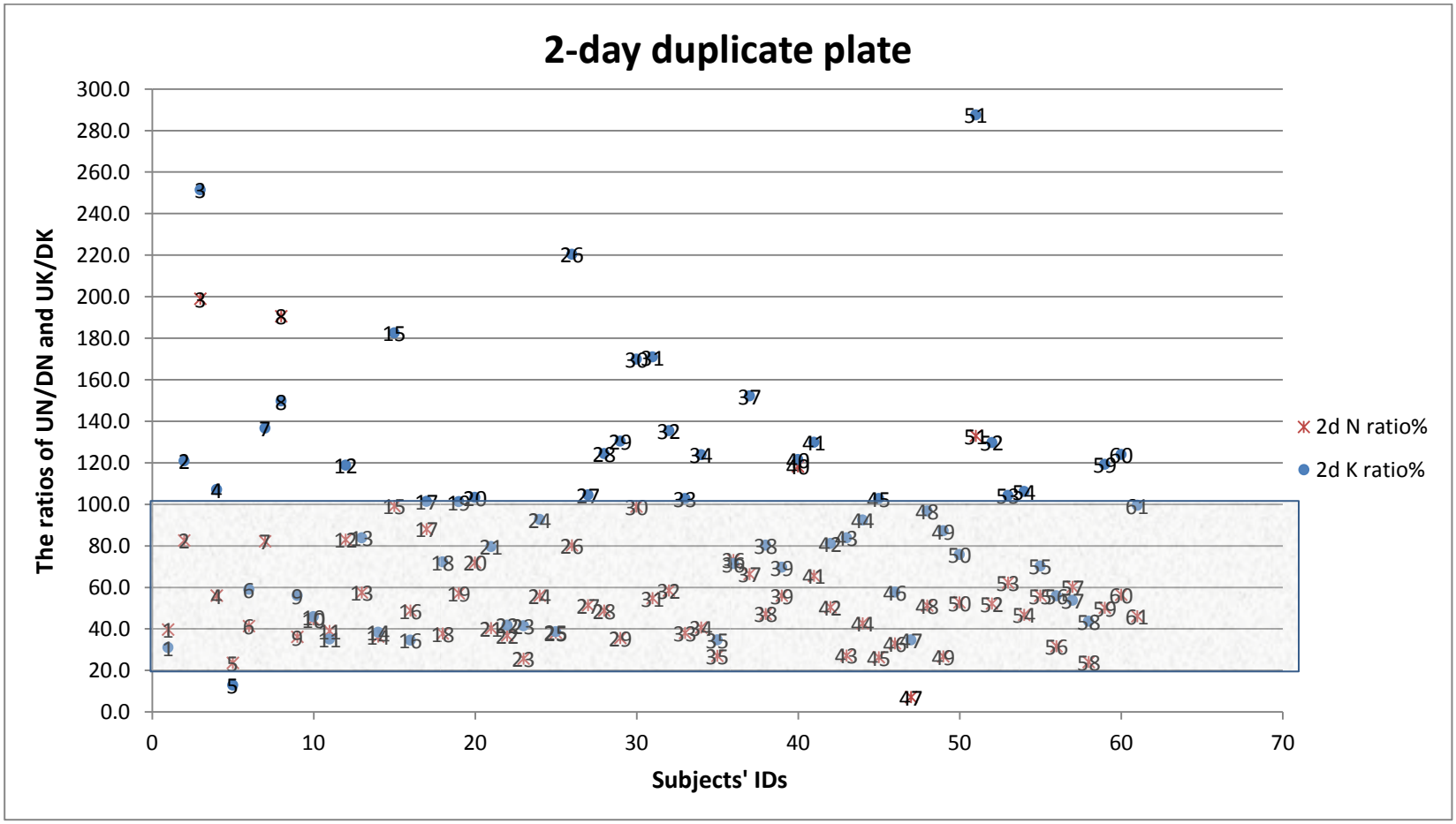


Figure 7.2 Distribution of individual's UN/DN and UK/DK ratios based on dietary data from the 2-day duplicate plate method



A summary of the results of UN/DN and UK/DK ratios and the number of valid as well as invalid data based on each criterion for both methods has been presented in Table 7.7.

Between the two methods there was only one child (number 45) whose dietary data was invalid in both methods.

Table 7.7 Summary of all valid and invalid data for both dietary methods

Method	Number (%) of children provided					
	Valid UN/DN & UK/DK	**Valid UN/DN & K intake**	**Valid UK/DK & N intake**	**Total (valid)**	**under reported data**	**over reported data**
3-day food diary	48 (79%)	6 (10%)	4 (6%)	58 (95%)	0	3 (5%)
2-day duplicate	30 (49%)	15 (24%)	1 (2%)	46 (75%)	15 (24%)	0

7.4.4. Other validity criteria for food diaries

The results of validity of the 3-day food diary method based on EI and PAL are presented in Table 7.8. According to the results, PAL for 12 children was below the cut-off point of 1.28. EI of three children was higher than the EI reported in NDNS for the same age group, while only one child had a lower EI than the reported for that age group.

Table 7.8 Number of children under, within and over the cut-off points for PAL and EI when dietary data collected by 3-day food diary method

Reporting category	EI	PAL
The number of children:		
- under cut-off point	1	12
- within the range	57	49
- over cut-off point	3	0

To examine the effect of over-reporters on the mean fluoride intake by food diary method, the three over-reporters were excluded from the analysis and the results before and after the exclusion were compared (Table 7.9). No, significant

difference was observed in the mean fluoride intake (mg) per day and on body weight basis. Consequently these subjects were not excluded from the study.

The exclusion of under-reporters for the duplicate method also did not greatly change the results of fluoride intake per day and per kg body weight (Table 7.9).

Table 7.9 Results of F intake before and after exclusion of over and under-reporters for the 3-day food diary and the 2-day duplicate methods

Dietary assessment method	Mean (SD) of fluoride intake	
3-day food diary Number of children	**Before exclusion** 61	After exclusion 58
mg/d mg/kg bw/d	0.533 (0.318) 0.025 (0.016)	0.532 (0.322) 0.025 (0.016)
2-day duplicate Number of children	**Before exclusion** 61	**After exclusion** 46
mg/d mg/kg bw/d	0.583 (0.263) 0.028 (0.013)	0.594 (0.254) 0.027 (0.016)

7.5 Discussion

7.5.1. Analytical methods

In this study the mean difference (bias) from test to re-test ranged from 0.003 to 0.017 µg for samples analysed by both direct and HMDS acid-diffusion method. The measurement error (typical variability) from test to re-test was also negligible indicating the reliability of the fluoride analytical tests. In addition the range of recovery recorded in the current study from 98.7% to 100% was in agreement with previously reported means of 98% (Maguire *et al.*, 2007, Zohouri & Rugg-Gunn 2000b), >99% (de Almeida *et al.*, 2007) and a range of 93-103% reported by Franco et al. (Franco *et al.*, 2005a).

7.5.2. Urine samples

Fluoride excretion in urine has been known as the most reliable, non-invasive biomarker of recent fluoride exposure. The rate of fluoride excretion varies throughout the day and night and this is dependent on the time of fluoride ingestion. Therefore, the use of 24-h urine samples has been recommended by the WHO (1999) to cover as much of the 24-h fluoride intake as possible.

However, it might be difficult to obtain complete 24-h urine samples as parents who are responsible for collecting the urine samples might forget to collect urine from one or more voiding which could result in underestimation of fluoride excretion. It is therefore important to check the completeness of 24-h urine samples.

Several markers as described in Chapter 2 section 2.3 have been used to check the completeness of 24-h urine samples. External marker such as PABA has been used for checking the completeness of 24-h urines in studies with adults (Bingham *et al.*, 1992, 1997, Bingham 2003) and children (Bates *et al.*, 2010). However, it is difficult to persuade parents to give the tablets to children as well as children may refuse to take tablets.

Consequently, to minimise burden on participants, the internal markers such as urinary creatinine excretion and urine flow rate (which are naturally occurring markers) have been suggested for use in validating the urine samples of children. Few 24-h urinary creatinine excretion reference values have been established for children. These references were outlined in Chapter 2 section 2.3. All the references except the WHO (1999) reference were based on height and/or weight since the major determinant of urine creatinine is muscle mass. Because skeletal muscle mass is also a major component of fat free mass, a strong age dependency of absolute urinary creatinine excretion is expected in growing children as creatinine excretion per unit of fat free mass steadily increases with age (Neubert & Remer 1998). It is known that cooked meat and heat-treated milk contain a considerable amount of creatinine and this is excreted promptly in urine after ingestion (Heymsfield *et al.*, 1983). In addition, creatinine which is present in all meats, increases the size of the whole body creatinine pool which in turn is proportional to the output of creatinine in urine (Neubert & Remer 1998). There is a difference both, between and within individuals in meat consumption and from day-to-day. Significantly less creatinine is excreted daily from individuals that eat a vegetarian diet when compared to individuals that eat a non-vegetarian diet. Based on these variations in the type and quantity of food, the reference range of 8-22 mg/kg bw/d suggested by Tietz (Tietz 1995) was selected to cover a daily variation in meat consumption and the growth rates of individuals.

On average the mean creatinine excretion of 13 mg/kg bw/d for children in the current study was within the Tietz reference range. However, the urinary

creatinine excretion values of 10 and 9 children was not within the reference range when dietary data were collected by the 3-day food diary and the 2-day duplicate methods respectively.

As suggested by Remer (Remer *et al.,* 2002) exclusion of 24-h urine samples should not be based on one criterion only. The second criterion used in this study was urine flow rate. A urinary flow rate of less than 5 ml has been suggested as incomplete by the WHO, while it is suspected as being diluted with water if it is >420 ml/h. The urine flow rates in this study ranged from 6.2 to 54.2 ml/h which were within the range suggested by WHO. In this study the mean urine flow rates of 20 and 21 ml/h measured for urine samples collected along with dietary data collection by 3-day food diary and 2-day duplicate methods respectively was similar to the figures of 21, 20 and 22 ml/h reported for 6-7 year old British children (Maguire *et al.,* 2007) but lower than 25 ml/h reported for 3-4 year old Swiss (Marthaler *et al.,* 2000) and 24 ml/h reported for 3-6 year old German children (Haftenberger *et al.,* 2001). However, they were higher than 17 m/h reported for 3-5 year old Venezuelan children (Acevedo *et al.,* 2007).

Overall, all collected 24-h urine samples in this study were considered valid according to the WHO criteria. This could be due to the close and frequent contact of the researcher with the parents, in which the parents were carefully instructed about the importance of the complete 24-h urine collections. In addition parents were interviewed after the collection period to confirm the completeness of the collection and the accuracy of the recorded times.

7.5.3 Validation of dietary assessment methods using biomarkers of urines

Accurate estimates of the dietary intakes of individuals are required for nutritional survey and epidemiological studies. However, assessing the validity and reliability of dietary methods is challenging. An independent verification of the validity of dietary methods should be conducted and built into the protocol of any dietary assessment method in order to obtain accurate information. Biological markers which are independent of the methods and habitual intakes can provide objective validation of dietary assessment methods (Bingham 2002) and have been used in several studies. However, there is no study in the literature that reports the use of biological markers of diet in validating dietary methods used in children.

7.5.3.1. Nitrogen and potassium

Urine nitrogen is a well-established and well-known biomarker of protein intake and has been used to investigate the validity of dietary assessment methods in adults. However, since urine nitrogen is derived from protein sources, the use of urine nitrogen as the only biomarker to adjust for nutrient intake other than protein such as fluoride could not be enough. For example in the UK diet, " meat and meat products", "cereal and cereal products", and "milk and milk products" are the three main sources of protein intake which contribute to protein intake at 36%, 23% and 16% respectively (Henderson *et al.*, 2003). Therefore, the use of an additional biomarker which presents in a greater variety of food and in almost similar quantities in different food groups is beneficial. Potassium is the nutrient which has been suggested to have this advantage since different food groups contribute almost equally to potassium intake. For example, the contribution of potato, fruit, meat and meat groups, cereal and cereal groups to total potassium intake is reported as 18%, 17%, 15% and 13% respectively (Tasevska *et al.*, 2006).

A range of urine to dietary nitrogen ratio (UN/DN) of 66-113% ((Porrini *et al.*, 1995, Black *et al.*, 2000, Black *et al.*, 1997, Bingham & Cummings 1985, Bingham *et al.*, 1995, Tasevska *et al.*, 2006).and UK/DK of 63-108% (Schashter *et al.*, 1980, Tasevska *et al.*, 2006) has been reported in adults. However, the available data using these two biomarkers in children are limited to balance studies (Voors *et al.*, 1983) or in relation to investigating the effect of urine electrolytes on blood pressure and bone density (Connor *et al.*, 1984, Jones *et al.*, 2001).

A study in the USA reported mean urine nitrogen of 2.12 g/d for 8-9 year old girls (Howat *et al.*, 1975). Mean urine nitrogen of 12.30 g/d was also reported in 10-12 year old boys in Japan (Nakagawa *et al.*, 1965). However, no urine to dietary nitrogen ratio was found in the literature for healthy children.

In children the reported dietary and urine potassium showed that urine to dietary potassium ratio ranged from 36% in 8 year old Tasmanian children (Jones *et al.*, 2001) to 86% in 13-15 year old US girls (Clark & Mossholder 1986).

However, none of these values were reported in relation to validation of dietary methods in healthy children.

The ratios of urine to diet for nitrogen and potassium (Figures 7.1 and 7.2) indicated that for the majority of children those ratios laid between 20 and 100% for both methods. This ratio might therefore be considered as an acceptable range for validation of dietary assessment methods. Due to the lack of a reference range for UN/DN and UK/DK ratios in children, other criteria were also considered for checking the validity of data in this study. Therefore, if the ratios for any child were not within the range of 20% to 100%, other criteria were used to avoid the wrong classification of dietary data to invalid or vice versa.

The results of this study indicated a greater tendency towards under-reporting when dietary data were collected by duplicate method. In general, food diaries have been regarded as the most accurate self-report methods for assessing dietary intakes (Bingham & Day 1997).

Several reasons were found in this study which might have contributed to over or under-reporting of dietary data. The size of the food diaries provided were small enough to be carried in the pocket or bag of the parents could have contributed to the increased number of valid data when compared with the 2-day duplicate method.

For duplicate method, the cost of duplicating food and drinks particularly when the participants ate out and had to purchase extra portion for duplicating could have contributed to under-reporting food intake. At the post collection interview, some parents addressed the lack of availability of enough food for duplicating which resulted in giving smaller portion sizes to their children. Some families changed their dietary and social behaviour during duplicating and avoided eating out. Others also mentioned that if there was not enough food to be duplicated, they encouraged the child to have something else which was sufficient to be duplicated. Changing dietary habits could be a major source of error in studies aiming to assess nutrient intakes based on habitual intakes. The possibility of errors in visual measurement of the duplicate portion could also contribute to under-reporting.

Whilst food diary method relies on food consumption tables and coding for the measurement of the nutrients, the duplicate method has the advantage that the actual diet of individuals is analysed directly without the use of food composition tables and therefore it is not affected by possible inadequate coding and non-inclusion of a given food type in the food consumption table (Basiotis *et al.*,

1987). In this study measures were taken to increase the accuracy of dietary data collection by both methods such as the post collection interview to ensure that all consumed food and drinks have been recorded or duplicated. In a study on 3-5 year old German children, duplicate collections were checked by requesting parents to keep a record of the duplicate food and drinks including the amount and the recipes (Haftenberger *et al.,* 2001). This measure, although improving the accuracy, could put an extra burden on parents to record and duplicate at the same time.

7.5.4 Further validation

Estimating energy intake (EI) and physical activity level (PAL) are also other measures which have been used by other investigators (Maguire *et al.,* 2007, Zohouri & Rugg-Gunn 2000b) to check the validity of the food diary method at group level.

In the present study although 12 children had a PAL ratio of less than a cut-off point of 1.28, the EI of only one child was below the lower limit reported in the NDNS survey. It can therefore be concluded that these children were not active. The use of EI and PAL for the validation of dietary assessment methods has some limitations. One limitations of assessment of EI is that it needs to be validated itself by comparing estimated data (EI) with measured energy expenditure. However, the reference method for assessing energy expenditure is doubly labelled water which is an expensive method. Another method used to validate EI is to compare the ratio of EI to measured or calculated BMR which is PAL (PAL=EI/BMR) with the suggested cut-off points for the population PAL (Torun 2005). However, the limitation of this approach is that the PAL cut-off points are valid only at the population level. Knowledge of individual's PAL should be acquired from completed physical activity questionnaire for each individual which is not practical in all studies (Black *et al.,* 2000). In addition, the accuracy of BMR is controversial as estimating BMR using Schofield (Schofield *et al.,* 1985) equation has been suggested to be less accurate compared with when it is measured (Torun *et al.,* 1996).

7.6. Conclusion

This study has provided for the first time unique information on validity of 3-day food diary and 2-day duplicate methods for estimating fluoride intake in children. From the results it can be concluded:

- Dietary intake and urinary excretion of both nitrogen and potassium could be used to check the validity of dietary assessment methods.
- Duplicate method was more likely to under-report dietary data whereas the possibility of over-reporting was greater when food diary method was used.
- There was a greater possibility of altering dietary habits by families when 2-day duplicate was used due to high cost of duplicating and lack of enough food.
- It is clearly inappropriate to attempt to validate estimates of diet with urine collections if the completeness of 24-h urine collections is not verified. Urinary excretion of creatinine and urine flow rate could therefore be used to check the completeness of 24-h urines.

Chapter 8 Assessment of dietary fluoride intake using two dietary methods "2-day duplicate plate" and "3-day food diary" and total daily fluoride intake

8.1. Introduction

This chapter presents the results of dietary fluoride intake estimated by "2-day duplicate" and "3-day food diary" methods and seeks to integrate the results into the existing literature. Sources of dietary fluoride intake, intake from toothpaste ingestion and total daily dietary fluoride intake estimated by each method have also been identified and presented. Finally, the results of total daily fluoride intake obtained from each method are considered in relation to recommendations for fluoride intake in young children.

8.2. Aim

This chapter aimed to estimate:

- fluoride intake from diet using "2-day duplicate" and "3-day food diary" methods
- total daily fluoride intake from diet and toothpaste ingestion

The objectives of the study were to:

- measure fluoride contents of food and drinks consumed by children,
- identify sources of dietary fluoride intake in children,
- estimate fluoride intake from toothpaste ingestion

8.3. Materials and methods

Methods of dietary data collection, expectorated saliva, toothpaste and rinse were explained in Chapter 5, sections 5.2.4.3 and 5.2.4.4

8.4. Results

The results on fluoride intake using each method have been presented in three sections:

i. The first section, presents the results on fluoride intake from diet
ii. The second section presents fluoride intake from toothpaste ingestion
iii. The last section describes total daily fluoride intake.

Section 1: Fluoride intake from diet

8.4.1. Daily dietary fluoride intake measured by 2-day duplicate method

On average, each child consumed 733g solid food, 328g drinks and 203 g water per day.

The mean (SD) and range of dietary fluoride intake per day (mg/d) and on body weight basis (mg/kg bw/d) for all children are presented in Table 8.1.

The contribution of all drinks (including water) was higher (58%) than food (42%) with a wide range from 0.046-0.846 mg/d.

Table 8.1 Mean (SD) and range of total dietary fluoride intake from food and drinks in all children [n=61]

F intake from:	mg/d		mg/kgbw/d	
	Mean (SD)	Range	Mean (SD)	Range
• Drinks	0.357 (0.221)	0.046-0.864	0.017 (0.011)	0.002-0.051
- drinking water	0.199 (0.206)	0.000-0.864	0.010 (0.010)	0.000-0.051
- other drinks	0.158 (0.173)	0.000-0.762	0.007 (0.008)	0.000-0.037
• Food	0.226 (0.202)	0.053-0.529	0.011 (0.005)	0.003-0.029
• Total	0.583 (0.263)	0.099-1.390	0.028 (0.013)	0.006-0.077

- **Daily dietary fluoride intake by social area**

The mean (SD) dietary fluoride intake in both social areas is presented in Table 8.2.

The point estimates of the mean daily dietary fluoride intake for children from the low social area (0.592 mg/d) and those from the high social area (0.570 mg/d) were almost identical. Besides, based on body weight both groups had similar intakes (0.028 mg/kg bw/d).

The contribution of drinks to daily dietary fluoride intake in both groups was similar compared with the contribution of food. Daily dietary fluoride intake from drinks was 0.371 (0.217) and 0.334 (0.229) mg/d for children from the low and the high social areas, respectively.

Table 8.2 Mean (SD) daily fluoride intake by social area [number of children]

	Mean (SD) daily F intake by social area			
Dietary sources of F intake	LSE [n=38]	%contribution	HSE [n=23]	%contribution
• Drinks (mg/d)	0.371 (0.217)	63	0.335 (0.229)	59
- drinking water	0.192 (0.188)	32	0.211 (0.235)	37
- other drinks	0.179 (0.178)	31	0.123 (0.161)	22
• Food (mg/d)	0.220 (0.100)	37	0.235 (0.105)	41
• Total				
- mg/d	0.592 (0.250)		0.570 (0.290)	
- mg/kg bw/d	0.028 (0.013)		0.028 (0.014)	

- **Daily dietary fluoride intake by gender**

Results on daily dietary fluoride intake for boys and girls are presented in Table 8.3. Total dietary fluoride intake was similar in both genders. The contribution of drinks to daily dietary fluoride intake was almost identical for both genders.

Table 8.3 Mean (SD) daily fluoride intake by gender [number of children]

	Mean (SD) daily F intake by gender			
Dietary sources of F intake	boys [n=35]	% contribution	girls [n=26]	% contribution
• Drinks (mg/d)	0.372 (0.209)	64	0.337 (0.238)	58
- drinking water	0.199 (0.181)	34	0.199 (0.238)	34
- other drinks	0.174 (0.178)	30	0.138 (0.166)	24
• Food (mg/d)	0.212 (0.102)	36	0.245 (0.100)	42
• Total				
- mg/d	0.584 (0.245)		0.582 (0.291)	
- mg/kgbw/d	0.027 (0.012)		0.029 (0.0150	

- **Daily dietary fluoride intake by age**

Daily dietary fluoride intake for different age groups is presented in Table 8.4. Dietary fluoride intake (mg) per day and per kg body weight was almost identical between the three age groups.

Table 8.4 Mean (SD) daily dietary fluoride intake by age groups [number of children]

Dietary sources of F intake	Mean (SD) daily F intake by age group		
	4 [n=20]	5 [n=22]	6 [n=19]
• Drinks (mg/d)	0.304 (0.230)	0.394 (0.242)	0.371 (0.180)
- drinking water	0.173 (0.184)	0.217 (0.248)	0.206 (0.179)
- other drinks	0.131 (0.163)	0.177 (0.205)	0.164 (0.144)
• Food (mg/d)	0.230 (0.122)	0.236 (0.093)	0.209 (0.089)
• Total			
- mg/d	0.534 (0.283)	0.630 (0.288)	0.580 (0.211)
- mg/kgbw/d	0.028 (0.015)	0.030 (0.014)	0.025 (0.010)

8.4.2. Daily dietary fluoride intake measured by 3-day food diary method

- **Fluoride concentration of consumed food/drink recorded in 3-day food diaries**

A total weight of 232.7 kg food and drink was consumed by children over the 3-day dietary collection period. On average each child consumed 838g of food, 293g of beverages and 139g of water per day. The fluoride content (µg/g) of 93% (216 kg) of all food and drink items consumed by these children had been previously analysed at Newcastle University. The remaining 7% consisted of 40 individual food and drink items which were measured in this project by the researcher. Table 8.5 presents the fluoride contents (µg/g) of these 40 items.

As expected, there was a wide range in the fluoride concentration of different food items. The highest fluoride concentration was obtained from sardines in oil (10.542 µg/g) while the lowest was obtained from peaches (0.020 µg/g). The results showed that when all the food items were grouped according to their compositions, the meat group contained the highest concentration of fluoride with a mean of 2.66 µg/g followed by fruit and vegetables, 0.279 µg/g.

Table 8.5 Weight of 40 food and drink items consumed (kg) and their fluoride concentration (µg/g) of these items which were analysed in this project

Food type	**Weight of food consumed (kg)**	**F con (µg/g)**
Drinks	**4.37**	**0.039**
Apple juice	3.70	0.060
Fresh orange juice	0.52	0.023
Mango juice	0.15	0.033
Milk and dairy products	**1.89**	**0.138**
Soya milk	0.36	0.307
Custard	1.16	0.024
Chocolate ice cream bar	0.37	0.082
Fruit and vegetables	**4.16**	**0.279**
Raw		
Fruit salad	0.23	0.021
Watermelon	1.80	0.036
Nectarine	0.30	0.031
Peach	0.16	0.020
Avocado	0.11	0.070
Cooked/processed		
Turnip	0.17	0.830
Green peas	0.50	1.314
Dahl	0.42	0.270
Potato curry	0.40	0.128
Canned peach	0.07	0.070
Cereal and cereal products	**3.51**	**0.167**
Semi sweet biscuit	0.17	0.042
Apple pie	0.19	0.113
Battenberg cake	0.18	0.260
Carrot cake	0.24	0.139
Halva semolina	0.18	0.020
Cereal chewy bar	0.26	0.080
Cheese & onion pasty	0.49	0.317

Continued Table 8.5.

Food type	**Weight of food consumed (kg)**	**F con (µg/g)**
Egg fried rice	0.37	0.346
Rice pudding	0.88	0.045
Candied popcorn	0.28	0.030
Mixed nuts	0.07	0.100
Fresh éclair	0.06	0.130
Dried apricot	0.04	0.040
Oat cake	0.04	0.680
Pistachio nut	0.06	0.160
Meat and meat products		
Chicken	**0.55**	**0.594**
Chicken casserole	0.34	0.549
Chicken pie	0.21	0.640
Red meat	**1.64**	**0.515**
Cooked lamb (stew)	0.80	1.050
Roast beef	0.32	0.580
Cornish pasty	0.42	0.151
Meat samosa	0.10	0.279
Fish	**0.40**	**6.866**
Sardine in oil	0.30	10.542
Herring in tomato sauce	0.10	3.190
Non-meat products		
Quorn mince	0.19	0.220

- **Daily dietary fluoride intake**

The mean (SD) and range of dietary fluoride intake (mg/day) and on body weight basis (mg/kgbw/d) for all children is presented in Table 8.6.

The contribution of food and drink (including drinking water) to daily dietary fluoride intake was fairly equal. Fluoride intake from drinks showed a wide range from 0.000 to 1.375 mg/d.

Table 8.6 Mean (SD) and range of total dietary fluoride intake from food and drinks in all children [n=61]

F intake from:	mg/d		mg/kgbw/d	
	Mean (SD)	Range	Mean (SD)	Range
• Drinks	0.266 (0.218)	0.000-1.376	0.013(0.011)	0.000-0.068
- drinking water	0.132 (0.173)	0.000-0.905	0.006 (0.009)	0.000-0.050
- other drinks	0.134 (0.194)	0.000-1.375	0.006 (0.009)	0.000-0.068
• Food	0.267 (0.183)	0.041-0.887	0.013 (0.008)	0.001-0.049
• Total	0.533 (0.318)	0.135-1.808	0.025 (0.016)	0.006-0.100

- **Sources of dietary fluoride intake in children**

The mean daily dietary fluoride intake (µg/d) and percentage of total fluoride intake from different food and drink items are presented in Table 8.7.

According to the results "Tap water" and "rice and pasta" had the highest contribution to total daily dietary fluoride intake (22%) followed by "squashes and cordials"(17%).

The next highest contributors to total daily dietary fluoride intake were from the food group: boiled vegetables (6.5%) and bread (5%). The contribution of black tea and soft drinks to total daily dietary fluoride intake was 3.5% and 2.5% respectively. The "other" group which consists of sweets, nuts, pickles, oils, puddings, cakes, raw vegetables, fruit and confectionary contributed to 6% of total daily dietary fluoride intake due to high frequency of consumption (1152 times).

Table 8.7 Details of sources of food and drinks recorded in food diaries, number of times these foods were consumed, fluoride intake from these foods and the contribution of each source to total daily dietary fluoride intake for all children

Food type	**Frequency of consumption (over 3-days)**	**Mean dietary F intake (µg/d)**	**Contribution to dietary F(%)**
All drinks	**493**	**266**	**48**
Water			
Bottled water	3	negligible	0
Tap water	187	132	22
Other drinks			
Black tea	10	19	3.5
Herbal tea	2	2	<1
Coffee/hot chocolate made with water	5	3	<1
Milk	246	4	<1
Squashes, cordials made with water	99	88	17
Soft drinks and juices from concentrate	137	14	2.5
Fresh fruit juice	5	negligible	0
Carbonated drinks including sparkling fruit juices	45	4	<2
All foods	**2410**	**267**	**52**
Cream, cheese and -yogurts	104	2	<1
Boiled vegetables	150	34	6.5
Rice & pasta	97	115	22
Bread and rolls	238	28	5
Breakfast cereal	131	5	1
Soups &gravy	12	4	<1
Canned soups	3	0.5	<1
Meat cooked with water such as casseroles	30	5	2
Grilled meat	31	1	<1
Processed meat	74	2	<1
Chicken and turkey	62	3	<1
Fish & seafood	58	28	6
Egg & egg dishes	22	0.4	<1
Other[β,]	1152	33	6

[β] includes: sweets, snacks, raw vegetables and fruit, pickles, nuts, preserves, oil and fat, puddings, desserts cakes, biscuits and confectionary

Dietary sources of fluoride were divided into 3 groups according to the source of water used for the preparation of the food and drinks:

- No added water: food and drinks with no added water for their preparation such as snacks and fresh fruit juice

- Manufacturer added water: food and drinks which manufacturers add water to prepare them such as processed meat and ready to drink fruit juices made from concentrate
- Customer added water: food and drinks which customers add water for their preparation such as home-made rice, pasta, and squashes.

The mean daily fluoride intake from each group is presented in Table 8.8.

According to the results, mean fluoride intake from the group with added water by customers is significantly greater (403.11 μg/d) than the other two groups. The food group with no added water for their preparation had the lowest contribution to total daily dietary fluoride intake (9%) despite having the highest frequency of consumption (1560 times).

Table 8.8 Mean daily dietary F intake according to the source of water for preparation

Dietary groups by source of water:	**Frequency of consumption (over three days)**	**Mean F intake (μg/d)**	**Contribution to dietary F (%)**
No added water	1560	48.72	9
Manufacturer added water	617	81.20	15
Consumer added water	405	403.11	76

- **Daily dietary fluoride intake by social area**

Mean (SD) dietary fluoride intake and the contribution of each dietary source to total daily dietary fluoride intake by social area is presented in Table 8.9.

The point estimate of the mean daily dietary fluoride intake for children from the high social areas (0.555mg/d) and the low social areas (0.519mg/d) was similar. The same trend was also observed when the daily dietary fluoride intake was expressed on body weight basis.

The contribution of dietary sources to daily dietary fluoride intake was also similar in both groups. Daily dietary fluoride intake in children from the high social area was 0.169, 0.098 mg/d from water and other drinks, respectively while

the corresponding data in children from the low social area were 0.109 and 0.156 mg/d respectively.

Table 8.9 Mean (SD) daily fluoride intake by social area [number of children]

Dietary source of F intake	Mean (SD) daily F intake by social area			
	LSE [n=38]		HSE [n=23]	
	Mean (SD)	% contribution	Mean (SD)	%contribution
• Drinks (mg/d)	0.265 (0.215)	51	0.268 (0.197)	48
- drinking water	0.109 (0.144)	21	0.169 (0.210)	30
- other drinks	0.156 (0.235)	30	0.098 (0.090)	18
• Food (mg/d)	0.255 (0.189)	49	0.288 (0.174)	52
• Total				
- mg/d	0.519 (0.312)		0.555 (0.335)	
- mg/kg bw/d	0.024 (0.014)		0.027 (0.018)	

- **Daily dietary fluoride intake by gender**

Mean (SD) dietary fluoride intake and the contribution of each source to total daily dietary fluoride intake for boys and girls are presented in Table 8.10. Total daily fluoride intake on the daily (mg/d) and body weight basis (mg/kgbw/d) in girls, (0.557 mg/d and 0.027 mg/kg/d) was almost identical with those of in boys (0.514 mg/d and 0.024 mg/kg bw/d).

The contribution of drinks and foods to daily dietary fluoride intake were similar in both genders.

Table 8.10 Mean (SD) daily dietary fluoride intake by gender [number of children]

	Mean (SD) daily F intake by gender			
Dietary sources of F intake	Boys [n=35]	% contribution	Girls [n=26]	% contribution
• Drinks (mg/d)	0.277 (0.233)	54	0.250 (0.201)	45
- drinking water	0.123 (0.133)	24	0.143 (0.218)	26
- other drink	0.154 (0.237)	30	0.107 (0.113)	19
• Food (mg/d)	0.237 (0.169)	46	0.307 (0.196)	55
• Total				
- mg/d	0.514 (0.293)		0.557 (0.355)	
- mg/kgbw/d	0.024 (0.013)		0.027 (0.018)	

- **Daily dietary fluoride intake by age**

Table 8.11 shows dietary fluoride intake in different age groups. Results indicated no substantial difference in dietary fluoride intake, either when expressed as mg/d or on body weight basis between three age groups.

Table 8.11 Mean (SD) daily dietary fluoride intake by age group [number of children]

Dietary sources of F intake	Mean (SD) daily F intake by age group		
	4 [n=20]	5 [n=22]	6 [n=19]
• Drinks (mg/d)	0.232 (0.204)	0.292 (0.288)	0.271 (0.276)
- drinking water	0.117 (0.197)	0.147 (0.180)	0.129 (0.143)
- other drinks	0.155 (0.235)	0.145 (0.286)	0.142 (0.130)
• Food (mg/d)	0.254 (0.189)	0.250 (0.137)	0.346 (0.215)
• Total			
- mg/d	0.441 (0.351)	0.543 (0.327)	0.617 (0.258)
- mg/kgbw/d	0.023 (0.019)	0.026 (0.016)	0.026 (0.010)

Section 2: Fluoride intake from toothpaste ingestion

8.4.3. Daily fluoride intake from toothpaste (data collected along with 2-day duplicate method)

Table 8.12 presents mean (SD) weight of toothpaste and water used by the children. The amount of fluoride ingested per brushing and per day is presented along with the percentage of fluoride ingested per brushing.

Mean (SD) weight of toothpaste loaded onto the toothbrush for all children was 0.69 g with a wide range from 0.16 to 3.00 g. The mean amount of fluoride ingested per brushing was 0.373 mg with a range from 0.020 to 2.324 mg. On body weight basis, children ingested 0.018 mg fluoride per kg with a range from 0.001 to 0.115 mg per brushing.

Most children (69%) claimed to brush their teeth twice per day. Based on the number of brushing per day, the amount of toothpaste ingested per day was found to range from 0.040 to 4.649 mg with a mean of 0.648 mg.

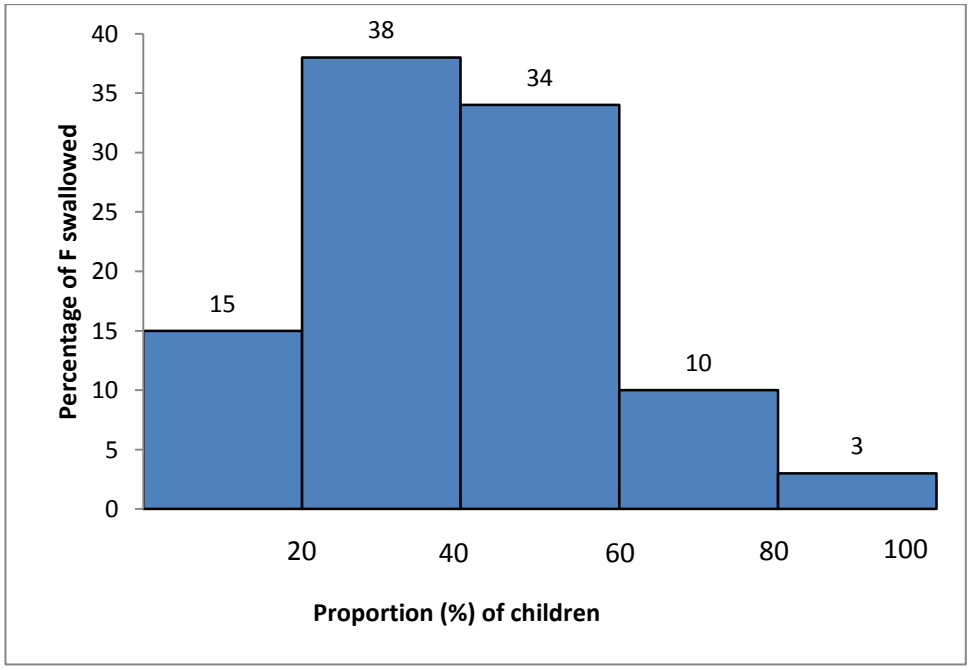
Based on body weight, the mean quantity of fluoride ingested per day was 0.032 mg per kg with a range from 0.002 to 0.230 mg.

Table 8.12 Toothpaste usage and ingestion by all children [n=61]

	Mean (SD)	Range
Weight of toothpaste on toothbrush (g)	0.69 (0.50)	0.16-3.00
Weight of water used for rinsing (g)	68.7 (14.6)	31.6-91.5
F ingestion(mg) per: - brushing - day	 0.373 (0.395) 0.648 (0.762)	 0.020-2.324 0.040-4.649
F ingestion (mg/kg bw)per: - brushing - day	 0.018 (0.020) 0.032 (0.040)	 0.001-0.115 0.002-0.230
Swallowed F/brushing (%)	42 (19)	6-85

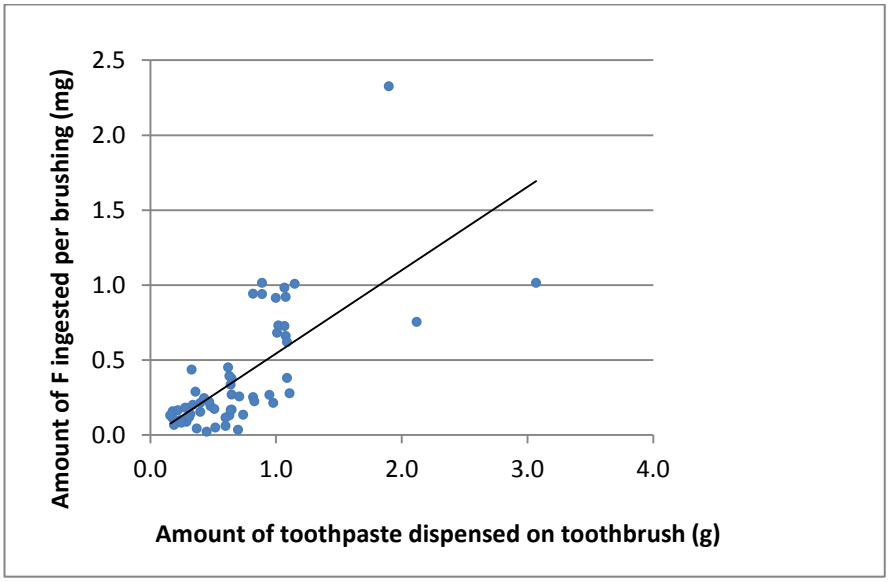
The percentage of fluoride swallowed per brushing had a wide range from 6-85% with a mean of 42%. The majority of children (38%) swallowed 21 to 40% of dispensed toothpaste while only 3% of children swallowed more than 80% of the toothpaste that was dispensed onto the toothbrush (Figure 8.1).

Figure 8.1 Proportion of children with different % of F swallowed per brushing



As shown in Figure 8.2, a positive, statistically significant correlation was found between the amount of used toothpaste (mg) and fluoride ingestion per brushing (mg) ($r=0.71$, $p<0.001$).

Figure 8.2 Correlation between the amount of toothpaste loaded (mg) on toothbrush and the amount of F (mg) ingested per brushing



- **Fluoride ingestion from toothpaste by social area**

The amount of toothpaste dispensed on the toothbrush and the amount of fluoride ingested per brushing by each social area is presented in Table 8.13. Results indicated that the mean (SD) weight of toothpaste used by children from the low social area was higher (0.81g) than children from the high social area. The point estimate of the mean ingested fluoride per brushing (0.428 mg) was also slightly higher in this group compared with the group from the high social area. Based on body weight, children from the low social area ingested more fluoride per brushing and per day. The percentage of the toothpaste swallowed by children between the two groups was almost identical (42% and 41% for low and high social areas, respectively).

Table 8.13 Toothpaste usage and ingestion by social area [number of children]

	LSE [n=38]		HSE [n=23]	
	Mean	Range	Mean	Range
Weight of toothpaste dispensed on toothbrush (g)	0.81 (0.56)	0.22-3.07	0.50 (0.29)	0.16-1.07
Weight of water used for rinsing (g)	68.14 (15.61)	31.67-91.58	69.81 (13.20)	37.17-89.09
F ingestion (mg) per:				
- Brushing	0.428 (0.448)	0.020-2.324	0.282 (0.273)	0.058-0.981
- Day	0.740 (0.881)	0.040-4.649	0.496 (0.490)	0.058-1.963
F ingestion (mg/kg bw) per:				
- Brushing	0.021 (0.022)	0.001-0.115	0.014 (0.016)	0.003-0.069
- Day	0.036 (0.045)	0.002-0.230	0.026 (0.031)	0.003-0.130
Swallowed F/brushing (%)	42 (21)	7-85	41 (16)	6-75

- **Fluoride ingestion from toothpaste by gender**

The amount of toothpaste dispensed on the toothbrush and the amount of fluoride ingested per brushing by gender is presented in Table 8.14. The weight of toothpaste used by boys and girls was similar. The amount of fluoride ingested per brushing and per day was almost identical for both boys and girls. Boys swallowed 44% of fluoride toothpaste that was loaded onto the toothbrush while the corresponding figure was 38% for girls.

Table 8.14 Toothpaste usage and ingestion by gender [number of children]

	Girls [n=26]		Boys [n=35]	
	Mean (SD)	Range	Mean (SD)	Range
Weight of toothpaste dispensed on toothbrush (g)	0.69 (0.59)	0.17-3.07	0.69 (0.43)	0.16-2.12
Weight of water used for rinsing (g)	71.23 (13.87)	37.17-91.58	66.94 (15.15)	31.67-90.80
F ingestion (mg) per:				
- Brushing	0.340 (0.334)	0.048-1.014	0.398 (0.438)	0.020-2.324
- Day	0.554 (0.574)	0.087-2.028	0.718 (0.878)	0.040-4.649
F ingestion (mg/kg bw) per:				
- Brushing	0.018 (0.019)	0.002-0.069	0.019 (0.021)	0.001-0.115
- Day	0.030 (0.037)	0.004-0.138	0.034 (0.043)	0.002-0.230
Swallowed F/brushing (%)	38 (18)	12-83	44 (19)	6-85

- **Fluoride ingestion from toothpaste by age**

According to results, the point estimate of the amount of toothpaste used by 5 year olds was slightly higher (0.72 g) than the other age groups (Table 8.15). However, on body weight basis, there was no substantial difference in the amount of fluoride ingested (mg) per brushing and per day.

The 4 year olds swallowed more fluoride (47%) per brushing compared with 5 (42%) and 6 (36%) year olds.

Table 8.15 Toothpaste usage and ingestion by age group [number of children]

	Age group,year		
	4 [n=20]	5 [n=22]	6 [n=19]
Weight of toothpaste dispensed on toothbrush (g)	0.67 (0.64)	0.72 (0.52)	0.68 (0.31)
Weight of water used for rinsing (g)	70.06 (12.79)	68.50 (14.40)	67.71 (17.27)
F ingestion (mg) per: - Brushing - Day	 0.406 (0.360) 0.691 (0.712)	 0.398 (0.503) 0.710 (0.986)	 0.308 (0.286) 0.530 (0.497)
F ingestion (mg/kg bw) per: - Brushing - Day	 0.022 (0.021) 0.038 (0.044)	 0.019 (0.025) 0.034 (0.048)	 0.013 (0.011) 0.022 (0.021)
Swallowed F/brushing (%)	47 (17)	42 (19)	36 (19)

8.4.4. Daily fluoride intake from toothpaste (data collected along with 3-day food diary method)

Table 8.16 presents mean (SD) weight of toothpaste and water used for brushing by the children, the amount of fluoride ingested per brushing, per day and percentage of fluoride ingested per brushing. Mean (SD) weight of toothpaste loaded onto the toothbrush for all children was 0.65g with a wide range from 0.11 to 1.50 g. The amount of fluoride ingested per brushing ranged from 0.007 to 1.379 mg, with a mean of 0.339 mg.

On body weight basis, the children ingested 0.016 mg fluoride with a range from 0.000 to 0.064 mg per brushing. Based on the number of brushings per day reported by children and their parents, the amount of toothpaste ingested per day was found to range from 0.010 to 1.838 mg with a mean of 0.568 mg.

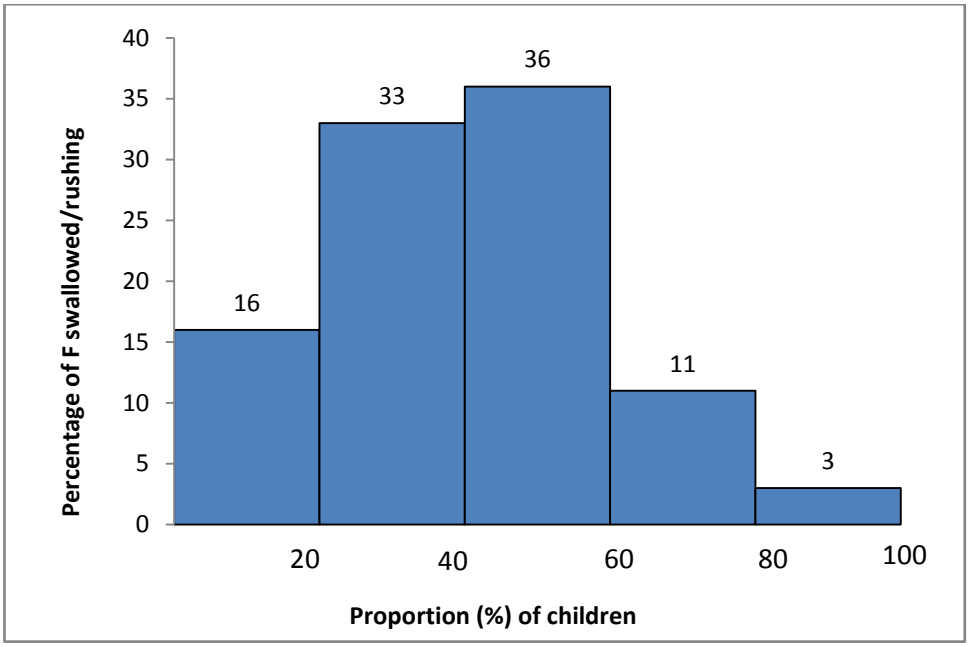
Based on body weight, the mean quantity of fluoride ingested per day was 0.027 mg with a range from zero to 0.088 mg.

Table 8.16 Toothpaste usage and ingestion of all children [n=61]

	Mean (SD)	Range
Weight of toothpaste dispensed on toothbrush (g)	0.65 (0.34)	0.11-1.50
Weight of water used for rinsing (g)	65.98 (16.24)	28.7-123.3
F ingestion(mg) per: - Brushing - Day	 0.339 (0.318) 0.568 (0.507)	 0.007-1.739 0.010-1.838
F ingestion (mg/kg bw) per: - Brushing - Day	 0.016 (0.013) 0.027 (0.023)	 0.000-0.064 0.000-0.088
Swallowed F/brushing (%)	41(19)	2-83

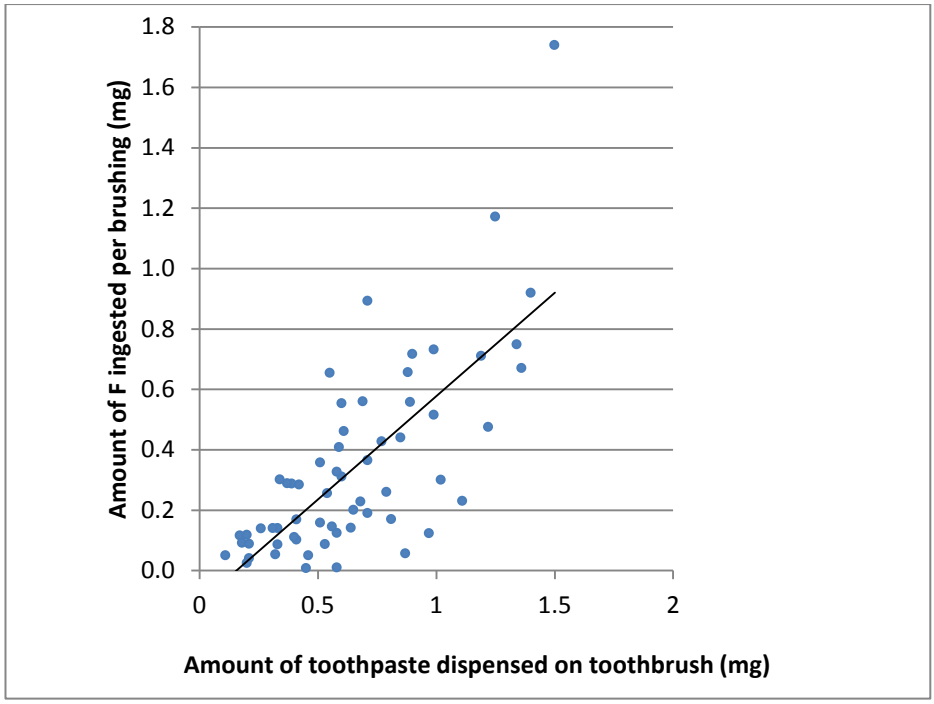
The percentage of fluoride swallowed per brushing for all children had a wide range from 2 to 83% with a mean of 41%. Almost 37% of children swallowed between 41-60% of dispensed toothpaste while 3% swallowed more than 80% of the dispensed toothpaste (Figure 8.3).

Figure 8.3 Proportion of children who swallowed different % of toothpaste per brushing



Fluoride ingestion from toothpaste was shown to be positively and strongly correlated with the amount of toothpaste used ($r=0.7$, $p<0.01$) as seen in Figure 8.4.

Figure 8.4 Correlation between the amount of toothpaste loaded (mg) and the amount of F (mg) ingested per brushing



- **Toothpaste fluoride ingestion by social area**

Table 8.17 presents the amount of toothpaste dispensed on the toothbrush and the amount of fluoride ingested per brushing by social area. Children from the low social area put more toothpaste on the brush (0.74g) and therefore the point estimate of the mean amount of ingested fluoride per brushing was slightly higher in this group (0.415 mg). The amount of fluoride ingested from toothpaste per day in children from the low social area was almost twice that in children from the high social area. Based on body weight, children from the low social area also ingested more fluoride per brushing (0.019 mg) and per day (0.031 mg).

The percentage of the toothpaste swallowed by children from the low social area was higher (44%) than those from the high social area (37%).

Table 8.17 Toothpaste usage and ingestion by social area [number of children]

	LSE [n=38]		HSE [n=23]	
	Mean (SD)	Range	Mean (SD)	Range
Weight of toothpaste dispensed on toothbrush (g)	0.74 (0.36)	0.20-1.50	0.49 (0.23)	0.11-0.97
Weight of water used for rinsing (g)	64.36 (17.36)	33.14-123.34	68.66 (14.16)	28.67-88.90
F ingestion (mg) per:				
- Brushing	0.415 (0.366)	0.010-1.739	0.215 (0.158)	0.007-0.654
- Day	0.679 (0.565)	9.830-1.838	0.384 (0.330)	0.014-1.308
F ingestion (mg/kg bw) per:				
- Brushing	0.019 (0.015)	0.000-0.064	0.010 (0.008)	0.000-0.032
- Day	0.031 (0.025)	0.000-0.088	0.019 (0.017)	0.000-0.064
Swallowed F/brushing (%)	44 (19)	3-84	37 (18)	2-81

- **Fluoride ingestion from toothpaste by gender**

The amount of toothpaste dispensed onto the toothbrush and the amount of fluoride ingested per brushing by gender is presented in Table 8.18. The weight of toothpaste used by boys and girls was fairly close. On body weight basis the amount of fluoride ingested per brushing and per day was almost identical for both genders (0.016, 0.015 mg/brushing and 0.030, 0.022 mg/kg bw/d in boys and girls respectively). In addition, boys swallowed 43% of fluoride loaded onto the toothbrush, while the corresponding figure was 38% in girls.

Table 8.18 Toothpaste usage and ingestion by gender [number of children]

	Girls [n=26]		Boys [n=35]	
	Mean (SD)	Range	Mean (SD)	Range
Weight of toothpaste dispensed on toothbrush (g)	0.64 (0.34)	0.17-1.50	0.66 (0.35)	0.11-1.40
Weight of water used for rinsing (g)	67.29 (18.94)	28.67-123.34	65.01 (14.13)	38.80-86.08
F ingestion (mg) per:				
- Brushing	0.329 (0.389)	0.007-1.739	0.347 (0.260)	0.010-0.919
- Day	0.463 (0.438)	0.014-1.739	0.646 (0.546)	0.010-1.838
F ingestion (mg/kg bw) per:				
- Brushing	0.015 (0.015)	0.000-0.064	0.016 (0.012)	0.000-0.044
- Day	0.022 (0.019)	0.000-0.064	0.030 (0.025)	0.000-0.088
Swallowed F/brushing (%)	38 (20)	2-81	43 (18)	3-83

- **Fluoride ingestion from toothpaste by age**

As presented in Table 8.19, older children swallowed less fluoride per brushing despite putting more toothpaste on the toothbrush. In general, no substantial difference was found between the amount of toothpaste used and fluoride ingested across the three age groups.

Table 8.19 Toothpaste usage and ingestion by age group [number of children]

	Age group		
	4 [n=20]	5 [n=22]	6 [n=19]
Weight of toothpaste dispensed on toothbrush (g)	0.51 (0.24)	0.66 (0.32)	0.78 (0.40)
Weight of water used for rinsing (g)	66.76 (13.64)	63.74 (14.87)	67.75 (20.37)
F ingestion (mg) per: - brushing - day	 0.269 (0.208) 0.458 (0.443)	 0.326 (0.242) 0.576 (0.458)	 0.429 (0.458) 0.675 (0.617)
F ingestion (mg/kg bw) per: - brushing - day	 0.014 (0.011) 0.024 (0.023)	 0.016 (0.012) 0.028 (0.022)	 0.017 (0.017) 0.028 (0.025)
Swallowed F/brushing (%)	42(17)	43(19)	36(20)

Section 3: Total daily fluoride intake

8.4.5. Total daily fluoride intake (2-day duplicate method)

As described earlier, children in the present study did not receive fluoride supplements. Therefore, diet and toothpaste ingestion were the only sources of fluoride intake. Mean (SD) total daily fluoride intake for all children is presented in Table 8.20. Results indicated that fluoride ingestion from toothpaste was almost equal to fluoride ingestion from all dietary sources.

Table 8.20 Mean (SD) and range of total daily fluoride intake from diet and toothpaste for all children [number of children]

F intake from:	mg/d		mg/kgbw/d	
	Mean (SD)	Range	Mean (SD)	Range
- Diet - Toothpaste	0.583 (0.283) 0.648 (0.762)	0.099-1.390 0.040-4.649	0.028 (0.013) 0.032(0.040)	0.006-0.077 0.002-0.230
- Total	1.231 (0.839)	0.299-5.550	0.060 (0.045)	0.019-0.275

- **Total daily fluoride intake by social area**

The point estimate of the mean total daily fluoride intake was slightly higher (1.331mg/d) in children from the low social area than those in the high social area (1.066 mg/d) (Table 8.21). However, on the body weight basis, total daily fluoride intake of children from both social areas was similar.

Table 8.21 Mean (SD) and range of total daily fluoride intake from diet and toothpaste by social area [number of children]

	F intake by social area			
	LSE [n=38]		HSE [n=23]	
	Mean (SD)	Range	Mean (SD)	Range
F intake (mg/d) from:				
- diet	0.592 (0.250)	0.099-1.220	0.570 (0.290)	0.234-1.390
- toothpaste	0.740 (0.881)	0.040-4.649	0.496 (0.491)	0.058-1.963
Total F intake (mg) per:				
- day	1.331 (0.938)	0.299-5.550	1.066 (0.630)	0.344-3.217
- kg bw per day	0.064 (0.049)	0.020-0.275	0.053 (0.038)	0.019-0.179

- **Total daily fluoride intake by gender**

Table 8.22 presents mean (SD) and range of total fluoride intake for boys and girls per day and on body weight basis.

The point estimate of the mean total daily fluoride intake in boys was slightly higher with a wider range than the corresponding data in girls. Nevertheless, when data were expressed on the body weight basis, no significant difference was observed between boys and girls.

Table 8.22 Mean (SD) and range of total daily fluoride intake from diet and toothpaste by gender [number of children]

	F intake by gender			
	Boys [n=35]		Girls [n=26]	
	Mean (SD)	Range	Mean (SD)	Range
F intake (mg/d) from:				
- diet	0.584 (0.245)	0.099-1.220	0.582 (0.291)	0.234-1.390
- toothpaste	0.718 (0.878)	0.040-4.649	0.554 (0.574)	0.087-2.028
Total F intake (mg) per:				
- day	1.302 (0.941)	0.299-5.550	1.136 (0.685)	0.344-3.217
- kg bw per day	0.061 (0.047)	0.020-0.275	0.059 (0.044)	0.019-0.179

- **Total daily fluoride intake by age**

Table 8.23 shows the mean (SD) total fluoride intake (mg) per day and per kg body weight for the different age groups.

Total daily fluoride intake based on body weight was similar in all age groups.

Table 8.23 Mean (SD) of total daily fluoride intake from diet and toothpaste by age [number of children]

	F intake by age		
	4 [n=20]	5 [n=22]	6 [n=19]
F intake (mg/d) from:			
- diet	0.534 (0.283)	0.630 (0.288)	0.581 (0.211)
- toothpaste	0.691 (0.712)	0.710 (0.986)	0.530 (0.497)
Total F intake (mg) per:			
- day	1.225 (0.885)	1.340 (0.001)	1.111 (0.494)
- kg bw per day	0.067 (0.053)	0.065 (0.052)	0.047 (0.021)

8.4.6. Total daily fluoride intake (3-day food diary)

Mean (SD) total daily fluoride intake for all children is presented in Table 8.24.

Results indicated that fluoride intake from toothpaste ingestion was almost equal to fluoride intake from all dietary sources.

Table 8.24 Mean (SD) and range of total daily fluoride intake from diet and toothpaste for all children [number of children]

	mg/d		mg/kg bw/d	
F intake from:	Mean(SD)	Range	Mean (SD)	Range
- Diet	0.533 (0.314)	0.135-1.808	0.025 (0.016)	0.006-0.100
- Toothpaste	0.568 (0.507)	0.010-1.838	0.027 (0.023)	0.000-0.088
- Total	1.101 (0.663)	0.238-3.329	0.052 (0.031)	0.012-0.165

- **Total daily fluoride intake by social area**

Total daily fluoride intake in children from both social areas was fairly close, 1.20 and 0.940 mg/d in the low and high social areas, respectively (Table 8.25). However, when expressed on the body weight basis (mg/kg bw/day), there was no substantial difference in total daily fluoride intake between the social areas.

Table 8.25 Mean (SD) and range of total daily fluoride intake from diet and toothpaste by social areas [number of children]

	F intake by social area			
	LSE [n=38]		HSE [n=23]	
	Mean(SD)	Range	Mean(SD)	Range
F intake (mg/d) from:				
- diet	0.519 (0.312)	0.135-1.543	0.556 (0.335)	0.146-1.808
- toothpaste	0.679 (0.565)	0.010-1.838	0.383 (0.330)	0.013-1.308
Total F intake (mg) per:				
- day	1.198 (0.748)	0.238-3.328	0.940 (0.461)	0.266-2.055
- kg bw per day	0.055 (0.033)	0.013-0.165	0.046 (0.024)	0.012-0.114

- **Total daily fluoride intake by gender**

The point estimate of the mean total daily fluoride intake for boys was slightly higher with a wider range from 0.238 to 3.329 mg/d compared with the corresponding data in girls (Table 8.26).

However, on a body weight basis, there was no significant difference in total daily fluoride intake between boys and girls.

Table 8.26 Mean (SD) and range of total daily fluoride intake from diet and toothpaste by gender [number of children**]**

	F intake by gender			
	Boys [n=35]		Girls [n=26]	
	Mean (SD)	Range	Mean (SD)	Range
F intake (mg/d) from:				
- diet	0.514 (0.293)	0.135-1.543	0.557 (0.355)	0.146-1.808
- toothpaste	0.646 (0.546)	1.010-1.838	0.463 (0.438)	0.014-1.739
Total F intake (mg) per:				
- day	1.160 (0.697)	0.238-3.329	1.021 (0.618)	0.286-2.838
- kg bw per day	0.053 (0.032)	0.012-0.165	0.049 (0.028)	0.016-0.114

- **Total daily fluoride intake by age**

Table 8.27 shows mean (SD) of total daily fluoride intake for different age groups.

Although, results indicated an increase in total fluoride intake per day with age, it was almost identical for all three age groups when expressed per kg body weight per day.

Table 8.27 Mean (SD) total daily fluoride intake from diet and toothpaste by age [nu,ber of children]

	F intake by age		
	4 [n=20]	5 [n=22]	6 [n=19]
F intake (mg/d) from:			
- diet	0.441 (0.351)	0.543 (0.327)	0.617 (0.258)
- toothpaste	0.458 (0.443)	0.576 (0.458)	0.675 (0.617)
Total F intake (mg) per:			
- day	0.899 (0.568)	1.119 (0.700)	1.292 (0.684)
- kg bw/day	0.047 (0.030)	0.054 (0.034)	0.053 (0.026)

8.5. Discussion

8.5.1. Fluoride intake

8.5.1.1. Dietary fluoride intake (2-day duplicate method)

Two day duplicate method is one of the dietary methods used for estimating dietary fluoride intake by many investigators (Table 8.28). In these studies dietary fluoride intake has been reported from food and all drinks (including drinking water) and/or total dietary fluoride intake without reporting from food and drinks.

In the present study, the parents were asked to collect food, drink and water separately to allow estimating the contribution and proportion of each source to dietary fluoride intake.

The results of this section are compared with those from fluoridated areas only which have also employed a duplicate plate method (Table 8.28). Since optimal salt fluoridation is comparable to optimal water fluoridation, results of this study were also compared with those studies conducted in regions where fluoridated salt was used.

Table 8.28 Summary of F intake studies used duplicate methods

Author, Date , Country	Age, years (number of children)	Source of F (ppm)	Mean (% proportion)* of dietary F intake [mg/d]			
			Water	Drinks	Food	Total
Guha-Chowdhury et al ,1996, Newzeland	3-4 (66)	Water (1)	n/a	n/a	n/a	0.360
Rojas-Sanchez et al, 1999, USA	1.5-3.3 (29)	Water (0.8-1.2)	n/a	0.396 (73%)	0.146 (27%)	0.541
Viall et al, 2000, Chile	3-5 (20)	Water (0.5-0.6)	n/a	0.415 (41%)	0.349 (35%)	0.766$^{\sigma}$
Grijalva et al, 2001, Mexico	8-9 (31)	Water (0.8)	1.61 (69%)	n/a	0.706 (30%)	2.31
Martinez-Mier et al, 2003, Mexico	1.5-3 (46)	Salt (250)	n/a	0.108 (16%) 0.112 (19%)	0.588 (84%) 0.521 (81%)	0.696 0.643
Franco et al, 2005, Colombia	1.8-2 (118)	Salt (180-220)	n/a	n/a	n/a	0.398
Rodrigues et al, 2009 in: Brazil (Bauru) Brazil (Brejo dos Santos) Lima, Peru Trujillo, Peru	4-6 (25) (21) (26) (25)	 Water (0.6-0.8) Water (0.6-0.8) Salt (180-200) Milk (1.0)	 0.34 (42%) 0.66 (63%) 0.04 (4%) 0.13 (11%)	 0.13 (16%) 0.15 (14%) 0.11 (12%) 0.39 (34%)	 0.33 (42%) 0.24 (23%) 0.75 (83%) 0.63 (55%)	 0.80$^{\sigma}$ 1.05$^{\sigma}$ 0.90$^{\sigma}$ 1.15$^{\sigma}$
Martinez-Mier et al, 2009	1.2-2.5 (12)	Water (1.0)	-	0.422 (76%)*	0.130 (24%)*	0.552
Present study	4-6 (61)	Water (0.9)	0.192 (31%)	0.158 (27%)	0.226 (42%)	0.583

* the proportion (%) has been estimated from the means

$^{\sigma}$ the means are estimated

- **Weight of food, drinks and water**

Children in this study consumed a higher amount of food (733g/d) than those reported by a study in Mexico City at 558g/d (Martinez-Mier *et al.,* 2003), while the amount of beverages consumed (701g/d) by children in Mexico City was almost twice the amount of that consumed by the children in the present study. A range from 596 to 711 g/d beverage consumption was also reported for children aged 2-8 years from Japan (Nohno *et al.,* 2006). Higher amount of beverages consumption was also found among 4-6 years old children from North Carolina, US (1048 ml/d) (Pang 1992).

The amount of water consumed that has been reported in a few studies ranged from 1136 g/d for 4 year olds in an Iranian study (Zohouri & Rugg-Gunn 2000a) to 1520 g/d for 4-6 year olds in a study of children from the USA (Ershow & Cantor 1989). Higher consumption of water (1568 to 2063 g/d) was reported for Mexican children living in 3 areas of Mexico with different fluoride concentration in their tap water (Grijalva-Haro *et al.,* 2001). Water consumption of children in the present study was almost one third of the amount reported for the Mexican, Iranian and US children. However, water consumption was fairly close to the corresponding amount reported by Rodrigues (Rodrigues *et al.,* 2009) for Peruvian children living in Lima (271g/d) and Trujillo (299g/d) whose sources of fluoride intake were salt and milk respectively.

However, a true comparison with other studies is difficult due to the differences in the age of children as well as the differences in dietary habits. Besides, the geographical location and temperature affect the amount of liquid consumption by children.

- **Dietary sources of fluoride intake**

Duplicate diets of food, drinks and drinking water were analysed to determine the proportion of fluoride derived from each of the dietary sources.

Mean fluoride intake of 0.226 mg/d from food in this study was higher compared with 0.130 and 0.146 mg/d reported in two US studies for 12, 15-30 months old (Martinez-Mier *et al.,* 2003) and 54, 16-40 months old children (Rojas-Sanchez *et al.,* 1999) (Table 8.26). Since optimal salt fluoridation is comparable to optimal water fluoridation, results from this study were compared with those reported in Mexico (n=46, 15-36 months old) where fluoridated salt is used and a much higher fluoride intake from food (0.588 , 0.521 mg/d) was reported for two

cities in Mexico (Martinez-Mier *et al.,* 2003). The difference in fluoride intake from food between this study and those from Mexico can be explained by the greater impact of salt on fluoride intake from food. Salt intake of 1-3 year old Mexican children was reported to be 1.9 g/d. This amount is equivalent to 0.475 mg/d fluoride intake if salt is fluoridated at 250 ppm.

With regard to fluoride intake from drinks, results of the present study for all drinks including water (0.357 mg/d) were lower than intake reported recently for the US children (0.422 m g/d) (Martinez-Mier *et al.,* 2009) and was three times higher compared with a Mexican population who used fluoridated salt (0.107 and 0.112 mg/d for Veracruz and Mexico City respectively) (Martinez-Mier *et al.,* 2003). The ingestion of fluoride from drinks in Mexico despite using fluoridated salt was attributed to the so called "*halo effect*" in that beverages were prepared in places where the water was fluoridated. These beverages were then distributed and consumed in areas where the water was negligibly fluoridated.

Fluoride intake from all drinks (including water) in this study was very close to that reported for children in fluoridated Indianapolis (0.396 mg/d) (Rojas-Sanchez *et al.,* 1999). In none of the above studies fluoride intake from water as a separate source has been reported. That could possibly be due to the lower water and higher beverage consumption by those children. Therefore, water was incorporated into drinks and fluoride intake was reported for both sources as a single source. However, depending on the liquid and water consumption habits among the study population, some studies have separated drinks from water. For example in one study on 1-3 year old Brazilian children living in an optimally fluoridated city (Bauru), powdered milk which had to be reconstituted by water was the major source of drink consumption (de Almeida *et al.,* 2007). Therefore, drinks were separated into two groups: water and milk together and other beverages. A very low fluoride intake from other drinks (0.07 mg/d) was reported in that study compared with 0.18 mg/d reported from milk and water suggesting the significant contribution of water to dietary fluoride intake among this group of Brazilian children.

When fluoride intake from water alone was considered, the results from the present study (0.199 mg/d) were much lower than that reported for Mexican children (1.61 mg/d) living in an optimally fluoridated area (Grijalva-Haro *et al.,* 2001). The higher intake of fluoride by children in Mexico reflected the higher

amount of water consumed due to high temperature. Fluoride intake from water by children in the current study was very close to that for Brazilian (0.18 mg/d) (de Almeida *et al.,* 2007) and Peruvian children living in Trujillo (Rodrigues *et al.,* 2009) whose sources of fluoride were water and fluoridated milk respectively. On the whole, fluoride intake from water and other drinks in the present study were very similar despite the fact that the amount of drinks consumed was higher than water. This suggested that most of the drinks consumed by children in this study were those diluted with water and therefore reflected the impact of fluoridated water on daily dietary fluoride intake. The higher contribution of drinks including drinking water (58%) compared with the contribution of the food (42%) to total dietary fluoride intake in this study supported this finding and was consistent with other studies which also reported a higher contribution (73% and 77%) from beverages (Rojas-Sanchez *et al.*, 1999, de Almeida *et al.,* 2007).

- **Total daily dietary fluoride intake**

Total fluoride intake from diet has been reported by a few duplicate diet studies (Table 8.28) which ranged from 0.36 to 2.32 mg/d (Guha-Chowdhury *et al.*, 1996, Grijalva-Haro *et al.,* 2001). The lowest dietary fluoride intake was reported for 3-4 year old children living in Newzeland (Guha-Chowdhury *et al.,* 1996) while the highest was for 8-9 year old Mexican children (Grijalva-Haro *et al.*, 2001).

Total dietary fluoride intake of 0.583 mg/d in this study was similar to that reported for the US children (n=12, age=15-30 months) (0.552 mg/d) (Martinez-Mier *et al.,* 2009) and British children (0.565mg/d) (n=6, age=6-7 years) (Maguire *et al.,* 2007). However, the results of dietary fluoride intake for British children was based on a 3-day food diary approach. The wide range in reported dietary fluoride intake in these studies suggested a variation in dietary habits, in the geographical locations as well as variation in the quantities of food and drinks consumed.

8.5.1.2. Dietary fluoride intake (3-day food diary method)

The number of studies that used 3-day food diary method was found to be limited to those for 4 year old Iranian (Zohouri & Rugg-Gunn 2000b), 15-30 months old

US (Martinez-Mier *et al.,* 2009) and 6-7 year old British children (Maguire *et al.,* 2007).

- **Fluoride contents of analysed food**

In this study food and drinks were categorised into subgroups (Table 8.5) according to their composition. Foods were subdivided into fruit and vegetables (raw and cooked), cereal and cereal products, meat and then other products. These categories were based on the preparation methods, whether they needed water for their preparation and whether they were prepared at home or by the manufacturer. In this study, fluoride concentration of ready-to-drink juices was found to be very low at 0.04 µg/g followed by milk and dairy products (0.1 µg/g). Among the food groups raw fruit and cereal products had the lowest fluoride concentration with the mean of 0.2 and 0.1 µg/g respectively, whereas that of for lamb stew was higher (1.06 µg/g) compared with the other foods that contained meat (e.g. Cornish pasty) which had a lower fluoride concentration (0. 15µg/g). This difference can be attributed to the impact of fluoridated water when it is used for cooking. Food such as rice, boiled vegetables and stews prepared with water therefore had a higher fluoride concentration in this study. Meat such as chicken and particularly fish are naturally high in fluoride. Fluoride concentration of canned fish has been reported to range from 0.19 (Cutrufelli *et al.*, 2004) to 40 (Wei & Hattab 1987) µg/g. In this study fluoride concentration of sardines was found to be 10.50 µg/g. The high fluoride concentration of fish in the present study was partly due to the inclusion of bones when it was prepared for the analysis. Since bones and shells accumulate fluoride, the inclusion of bones and shells contributes to the higher fluoride content of these foods. Similarly, inclusion of bones in chicken products contribute to the higher fluoride content of chicken products (Dolan *et al.,* 1978). A fluoride concentration between 0.6-10.6 µg/g has been reported for chicken products (Wiatrowski *et al.*, 1975, Singer & Ophaug 1979). Fluoride concentration of chicken casserole and chicken pie measured in the present study were 0.54 and 0.64 µg/g respectively. Chicken casseroles were home-made and analysed without bones, whereas chicken pie was ready-made and may have contained traces of bones.

- **Weight of food, drinks and water**

The amount of food, consumed by children in the present study (838g) was almost as twice as that was consumed by 4-year old Iranian children (Zohouri & Rugg-Gunn 2000b).

On average children in this study consumed 293 g/d drinks which was lower than the consumption of 626-701 g/d reported for 15-36 months old Mexican (Martinez-Mier *et al.,* 2003) and 596-711 g/d for 2-8 year old Japanese children (Nohno *et al.,* 2006). Water consumption in this study was 139 g/d which was considerably lower than the theoretical suggestion of 1600 g/d for 4-6 year old children (McClure 1943). Some other recent studies reported water intake of 1520 g/d for the US children (Ershow & Cantor 1989), 447 g/d for Iranian (Zohouri *et al*., 2000a) and 1568-2068 g/d for Mexican children (Grijalva-Haro *et al.,* 2001). Lower amounts of water consumption have been reported for 4-6 year old children from Peru (271, 299g/d) living in two cities of Lima and Trujilla (Rodrigues *et al.,* 2009) and 3-5 years old from Australia (200 and 209g in winter and summer respectively) (Crosby & Shepherd 1957).

Overall, the differences in the reported amount of water, drinks and food between this study and other studies can be attributed to differences in social custom, dietary patterns and climate temperature. However, it has to be noted that due to the age difference between children from these studies, it is difficult to compare the results of this study with any other available data.

- **Total daily dietary fluoride intake**

Total daily dietary fluoride intake for children in the present study using the 3-day food diary method was 0.533 mg which was comparable with the data reported for British, US, Iranian and Hungarian children (Table 8.29). Dietary data collection for Hungarian children differed from the current study because those data were recorded over a 7-day period. Total dietary fluoride intake for children in the present study was fairly similar to 0.565 mg/d reported for 6-7 year old British (Maguire *et al.,* 2007), 0.552 mg/d for 15-30 months old US children (Martinez-Mier *et al.,* 2009). However, it was lower than 0.698 and 0.720 mg/d reported for 4 year old children from Iran (Zohouri & Rugg-Gunn 2000b) and Hungary (Schamschula *et al.,* 1988) respectively.

The higher dietary fluoride intake in Iranian children could be due to the higher climate temperatures in Iran and consequently drinking more water by the

Iranian children. While for Hungarian children it could be attributed to the high consumption of soups prepared with fluoridated water. This food was the major component of the Hungarian diet. Therefore, dietary habits could be the main determinants of fluoride intake in different countries. On the body weight basis, dietary fluoride intake of 0.025 mg/d in the present study was the same as that reported for 6-7 year old British children living in an optimally fluoridated area (Maguire *et al.,* 2007).

- **Sources of dietary fluoride intake**

Daily dietary fluoride intake from food, drinks and water and total dietary intake has been reported in several studies which used a food diary approach with children living in optimally fluoridated area (Table 8.29). In these studies daily fluoride intake from food was reported at 0.38 mg/d for 3-4 years old Hungarian children (Schamschula *et al.,* 1988), 0.145 mg/d for 4 years old Iranian children (Zohouri & Rugg-Gunn 2000b), and 0.188 mg/d for the US children (Martinez-Mier *et al.,* 2009). Daily fluoride intake from food for children in the present study at 0.267mg/d was lower than the corresponding values for children in Hungary whereas it was higher than the values reported for children from Iran and the USA. One of the factors that could contribute to different levels of fluoride intake from food is the preparation method. Cooking food with fluoridated water at home could result in higher fluoride intake from this source. This could be the case for higher intake from food in both Hungarian and the current study. However, it has to be noted that ready-made food prepared in fluoridated areas could still contribute to substantial fluoride intake for populations living in both fluoridated and non-fluoridated areas.

With regard to daily fluoride intake from drinks excluding water, fluoride intake of 0.134 mg/d in the present study was lower than 0.299, 0.270 and 0.190 mg/d reported for 6-7 year old British (Maguire *et al.,* 2007) , 4 year old Iranian (Zohouri & Rugg-Gunn 2000b) and 3.9 year old Hungarian children (Schamschula *et al.,* 1988). The difference could be partly explained by the difference in the type of drinks consumed by the children. Drinks which are diluted with fluoridated water could have contributed to a higher fluoride intake compared with ready-to-drink beverages and fresh juices which were found to have lower fluoride concentration in the present study. Another explanation as

discussed earlier is the amount of drinks consumed because of the higher temperatures in different countries.

Daily fluoride intake of 0.132 mg/d from water in the current study was fairly close to 0.150 mg/d reported for Hungarian children (Schamschula *et al.,* 1988) but lower than 0.263 mg/d reported for 4 year olds Iranian children (Zohouri & Rugg-Gunn 2000a). A lower fluoride intake from water was reported for 6-7 year old British children (0.083 mg/d) compared with the fluoride intake from water by children in the present study. Similar fluoride intake from water and drinks in the present study could support that water by itself may not be the main source of fluoride intake in some children, yet it had a great impact when added to food and drinks for their preparation.

Diet including water can comprise up to 70% of total daily fluoride intake in children younger than 6 years old (Levy *et al.,* 2003). It is therefore important to determine the contribution of dietary components to total daily fluoride intake. Sources of dietary fluoride intake and the relative contribution of all dietary components to total daily dietary fluoride intake has been investigated by only a few studies (Levy 1995, Zohouri & Rugg-Gunn 2000a, Zohouri *et al.,* 2006, Levy *et al.,* 2003).

In this study, a 3-day food diary with interview and collections of home-made food and drink samples allowed the collection of more detailed qualitative and quantitative information such as the sources of fluoride. This information cannot be collected using other methods such as duplicate plate, since all food and drinks are pooled.

Limited data is available with regard to the sources of dietary fluoride in young children. A study of 4 years old children in Iran (Zohouri & Rugg-Gunn 2000a) and 6-7 years old children in the UK (Zohouri *et al.,* 2006) have identified the sources of dietary fluoride intake through the use of 3-day food diaries (Table 8.29). However, for other studies listed in Table 8.29, the contribution of dietary sources has been estimated from the mean fluoride intake from each dietary source.

The 26% contribution of drinks in the present study was similar to those reported for 4 year old Iranian (Zohouri & Rugg-Gunn 2000a) and Hungarian children (Schamschula *et al.,* 1988). However, it was lower than 41% reported for 6-7 year old British children (Zohouri *et al.*, 2006). Among drinks, those diluted with

water contributed at 31% for 6-7 year old British children (Zohouri *et al.*, 2006) which was almost as twice as 17% found in the present study. The lower contribution of this group of drinks could be partly attributed to the higher frequency of consumption of ready-made drinks by the children in the present study.

The 53% contribution of food in the present study was similar to that reported for Hungarian children but it was higher than 48% and 39% reported for 6-7 and 4 year old British and Iranian children.

The contribution of water to total dietary fluoride intake in this study was 21% which was lower than the corresponding values of 38% reported for 4 year old Iranian children (Zohouri & Rugg-Gunn 2000a). Moreover, the contribution of water to daily fluoride intake in the present study was higher than that reported in 2006 for 6-7 year old children living in the same locality as the children in the present study (Zohouri *et al.*, 2006).

In order to investigate the relative importance of the home supply of water to the total dietary fluoride intake, foods and drinks were divided into subgroups based on the use of water in their preparation (Table 8.8). The results showed that the contribution of the customer added water group was considerably higher (76%) than the contribution of the manufacturer-added water group (15%). Food such as “rice and pasta” and “boiled vegetables” which were mainly prepared at home, contributed 22% and 6.5% respectively. Rice and pasta has been previousely reported to contribute 16% to dietary fluoride intake of British children (Zohouri *et al.*, 2006). The findings of the present study and those by Zohouri et al. supported the impact of fluoridated water on total daily dietary fluoride intake in optimally fluoridated area.

Table 8.29 Studies of dietary fluoride intake using food records [%contribution of each source]

Author, date, Country	Age (yr)	F con of water (ppm)	Mean fluoride intake from all dietary sources (mg)		
			Drinks	Drinking water	Food
Schamschula et al. 1988, Hungary	3.9	0.5-1.1	0.190 [26%]	0.15 [21%]	0.38 [53%]
Zohouri et al. 2000, Iran	4	0.6	0.270 [23%]	0.263 [38%]	0.145 [39%]
Zohouri et al. 2006, UK	6-7	≥0.7	0.299 [41%]	0.083 [11%]	0.209 [48%]
Martinez-Mier et al. 2009, USA	1.5-2.5	1	0.316*[~63]	-	0.188 [~37]
Present study, UK	4-6	0.97	0.134 [26%]	0.132 [22%]	0.267 [52%]

* water was included

8.5.2. Fluoride intake from toothpaste ingestion (both collections)

- **Toothpaste usage**

Results of the present study indicated that on average children used 0.69 g (in both collections) toothpaste with a wide range from 0.16g to 3g (2-day duplicate) and 0.11 to 1.5 g (3-day food diary). Children in the current study on average used larger quantity of toothpaste compared with other studies and the recommended amount of 0.25 g (DoH/BASCD 2009). Only 15% (2-day duplicate) and 13% (3-day food diary) of children used 0.25g recommended for this age group. Almost half of the children used more than 0.60 g toothpaste in both collections. The greater usage of toothpaste by children can be attributed to the lack of knowledge on the use of fluoridated toothpaste among parents and children and unsupervised brushing while children apply toothpaste on the brush. Previously reported mean values for the amount of toothpaste used per brushing by children aged 2 to 7 years have been fairly consistent over the past 20 years: 0.45 g for 4 year old Canadian (Naccache *et al.*, 1992), 0.36 g for 30-month-old English (Bentley *et al.*, 1999), 0.43 g for 4-7-year old Brazilian (Pessan *et al.*, 2003), and 0.49 g for 1-3 year Brazilian children (de Almeida *et al.*, 2007). In a comprehensive study of fluoride ingestion from toothpastes (0 – 1500 µg F/g) by children aged 1.5 – 4.6 years in 7 European countries, (Cochran *et al.*, 2004a), it was found that the mean amounts of toothpaste used per brushing during a home

visit were 0.36, 0.41 and 0.49 g for the age ranges 1.5-2.5, 2.5-3.5 and > 3.5 years, respectively. The average amount of toothpaste used in the present study was higher compared with the findings from previous studies.

- **Toothpaste ingestion**

The results on the quantities of fluoride ingested per brushing in the present study at 0.373 mg (2-day duplicate method) and 0.339 mg (3-day food diary method) was lower than 0.42 , 0.48 and 0.59 mg/brushing reported for 30-months old children from the North West region of England (Bentley *et al.*, 1999), 6-year olds living in a fluoridated area in the north-east of England (Maguire *et al.*, 2007) and 1-3 year old Brazilian children respectively (de Almeida *et al.*, 2007). However, the amount ingested per brushing in this study was higher than the 0.13 mg reported for 4-5 year old Malaysian children (Siew Tan & Razak 2005) and 0.26 mg/brushing reported for 4-7-year-old Brazilian children (Pessan *et al.*, 2003).

The factor which had the largest effect on fluoride intake was the amount of toothpaste dispensed. A positive significant correlation between the amount of toothpaste used and the amount of fluoride ingested per brushing was found in both collections in the present study which confirmed a direct relationship between the two parameters. This positive correlation was also reported for 1-3 and 4-7 year old Brazilian children (de Almeida *et al.*, 2007, Pessan *et al.*, 2003, Paiva *et al.*, 2003). The findings of the present study also showed an inverse correlation between fluoride intake per brushing and age which was in agreement with the findings for Canadian children (Osuji 1988, Naccache *et al.*, 1992). The results of these studies clearly showed a greater tendency in younger children to swallow more toothpaste as they might not have learned to control their swallowing reflections.

On a body weight basis, the average fluoride intake from toothpaste ingestion by children in the present study was 0.032 (0.040) and 0.027 (0.023) mg/d along with 2-day duplicate and 3-day food diary methods respectively. On the basis of applying 0.55 g of a toothpaste containing 1000 µg F/g and brushing once per day the mean ingested fluoride was estimated and reported to be 0.018, 0.013 and 0.009 mg/kg bw/day for children aged 2-3, 4 and 5 years old respectively (Fomon *et al.*, 2000). In this study children used higher amount of toothpaste (0.69g) containing 1000 µg F/g and ingested 0.016 and 0.018 mg F/kg

bw/brushing. This was slightly higher than the estimated amounts reported by Fomon et al. for 4 and 5 year olds.

A mean value of 0.06 (0.03) mg F/kg bw/day has been reported for children aged 30 months from the north-west of England who used a 1450 µg F /g toothpaste (Bentley *et al.*, 1999).

Children in this study despite using a higher amount of toothpaste with a mean concentration of 1000 µg F/g, and higher frequency of brushing (69% brushed twice daily) ingested less fluoride.

8.5.3. Total daily fluoride intake (2-day duplicate method)

In this study, no child took any types of fluoride supplement. Toothpaste and diet were the only sources of fluoride intake in these children. Studies reported the mean total daily fluoride intake from diet and toothpastes have been presented in Table 2.10 in the literature review. However, those studies used duplicate method have been summarised in Table 8.30.

Total daily fluoride intake of children in the present study on the basis of body weight was similar to those reported for 4-6 year old Brazilian (Pessan *et al.*, 2003) and 16-40 months US children (Rojas-Sanchez *et al.*, 1999). Total daily fluoride intake of 22-45 month olds Colombian (Franco *et al.*, 2005a) and 1-3 year old Brazilian children (de Almeida *et al.*, 2007) was almost as twice as the amount found in children of the present study (Table 8.30).

The importance of fluoride ingestion from toothpaste has been highlighted in many studies. Contribution of 70% and 64% from toothpaste to total fluoride intake has been reported for Colombian children aged 22-35 months (Franco *et al.*, 2005a) and 15-36-month-old Mexican children respectively (Martinez-Mier *et al.*, 2003). For a group of Brazilian children living in areas where water is fluoridated at a level of 0.7 mg/l a large contribution of 58% to 69% from toothpaste was reported (Paiva *et al.*, 2003). However, lower contribution of 29% for 3-6 year olds German (Haftenberger *et al.*, 2001), 22% for 6 year olds US (Iowa) (Levy *et al.*, 2003), 47% for 3-4 and 6-7-years old children from New Zealand and England respectively (Guha-Chowdhury *et al.*, 1996, Maguire *et al.*, 2007), and 39% for 16-40-month-old children from Indianapolis, USA (Rojas-Sanchez *et al.*, 1999) was reported from toothpaste. The percentage contribution of toothpaste to total daily fluoride intake (53%) among children in the present

study was higher than the above values. The difference in reported contribution of toothpaste to total daily fluoride intake could be explained in part by the difference in ages of the children in Colombia (aged 22-25 months) and Brazil (aged 1-3 years) who were younger than the children in the present study and there was a greater tendency to swallow toothpaste as they may have not learned to expectorate properly. However, the lower contribution of toothpaste to total daily fluoride intake among 16-40 month olds children from Indianapolis was attributed to the use of a small amount of toothpaste and less frequency of brushing per day (once per day).

The 47% contribution of diet to total daily fluoride intake in the present study, was lower than 75% and 61% reported for 3-5 year old Chilean (Villa *et al.*, 2000) and 16-40 months old US children respectively (Rojas-Sanchez *et al.*, 1999). Nevertheless, it was higher than 20% and 30% reported for 1-3 year old Brazilian (de Almeida *et al.*, 2007) and 22-35 months old Colombian children, (Franco *et al.*, 2005a).

The distribution of children ingesting fluoride at levels below, within or above the optimum level is demonstrated in Figure 8.5.

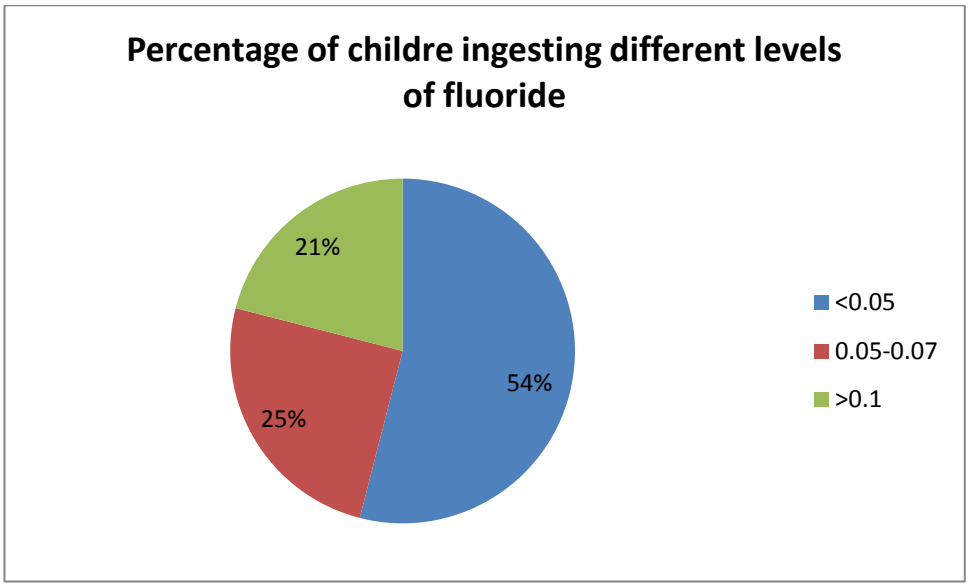
Total daily fluoride intake of more than half of the children was below the optimal range of 0.05-0.07 mg/kg bw/d. While a quarter of children in the present study received fluoride within the recommended level, one third ingested fluoride above 0.1 mg/kg bw/d.

Table 8.30 Summary of studies on total daily fluoride intake

Author (year)	Age (yrs)	F sources (mg/kg bw/d)		Mean (SD) total F intake
		Diet, mean (Contribution%)	Toothpaste, mean (Contribution%)	mg/kg bw/d
Rojas-Sanchez[α] (1999)	1.5-3.3	0.040 (61%)	0.028 (39%)	0.070 (0.007)
Pessan (2003)	4-7	0.018 (43%)	0.037 (57%)	0.056 (0.040)
Paiva (2003)[δ]	1.6-3.1	0.027 (36%) 0.040 (45%)	0.061 (64%) 0.052 (55%)	0.088 (0.049) 0.090 (0.022)
Franco (2005)	1.8-2	0.040 (30%)	0.107 (70%)	0.110 (0.090)
de Almeida (2007)	1-3	0.025 (18.5%)	0.106 (81.5%)	0.130 (0.087)
Present study	4-6	0.028 (47%))	0.032 (53%)	0.06 (0.045)

[α] Reported values for fluoridated Indianapolis , [δ] reported for two fluoridated cities Ibia and Piracicaba

Figure 8.5 The percentage distribution of children receiving fluoride based on daily recommendation of "0.05-0.07" mg/kg bw/d



8.5.4. Total daily fluoride intake (3-day food diary)

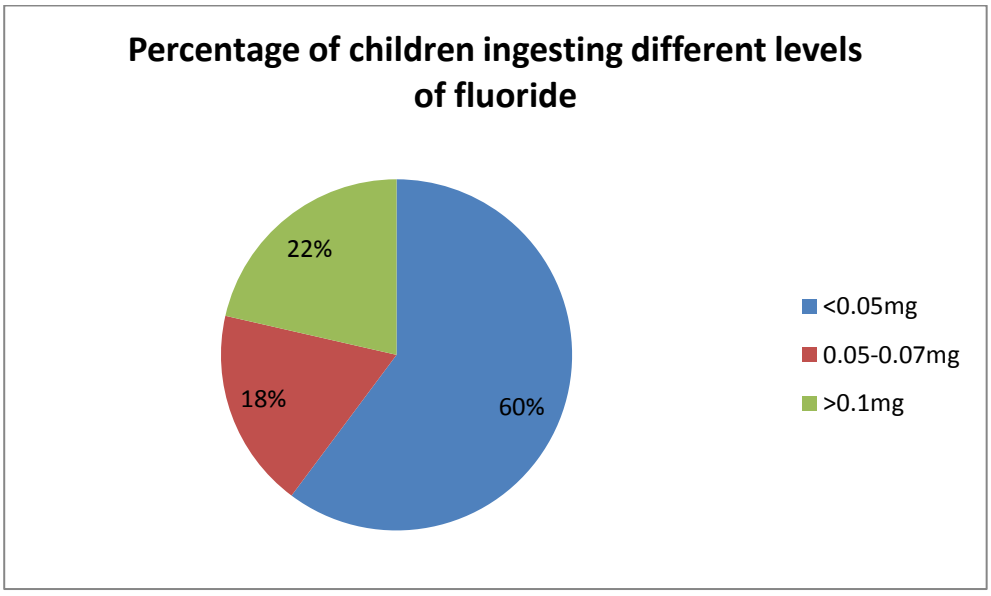
There were only two studies to which the results of the current study could be compared with and those were the studies conducted in Iran (Zohouri & Rugg-Gunn 2000b) and England (Maguire *et al.*, 2007) which used food diary method. The mean total daily fluoride intake of children in this study was 0.05 mg/kg bw/d and that was higher than the corresponding value of 0.03 mg/kg bw/d reported for 4 years old Iranian children (Zohouri & Rugg-Gunn 2000b) but

similar to 0.047 mg/kg bw/d reported for 6-7 year old British children (Maguire *et al.*, 2007).

Toothpaste ingestion contributed to 52% of total daily fluoride intake in this study which was slightly higher than that reported for 6-7 year British children (47%) (Maguire *et al.*, 2007).While for the Iranian children a contribution of toothpaste reported at 25% (Zohouri & Rugg-Gunn 2000b).

The mean total daily fluoride intake of children in the present study at 0.05 mg/kg bw/d was within the range of 0.05-0.07 mg/kgbw/d regarded as the "optimum" range of daily fluoride intake (Burt 1992). However, on individual basis, only a small proportion (18%) of children in this study received fluoride within the recommended range. For the majority of children (60%) total daily fluoride intake fell below the optimal range. One third of children ingested daily amount above 0.1 mg/kgbw/d (Figure 8.6).

Figure 8.6 The percentage distribution of children receiving fluoride based on daily recommendation of "0.05-0.07" mg/kg bw/d



8.5.5. Total daily fluoride intake between social areas, genders and age groups (both collections)

In this study the sample size was calculated to address the main aim of the study which was to compare dietary assessment methods used to estimate fluoride intake. Therefore, the sample size did not allow investigating statistical differences in fluoride intake from diet (estimated by each dietary assessment

method), toothpaste and total daily fluoride intake between genders, social areas and age groups. However, the descriptive data showed that based on the body weight no mathematically substantial difference was found in the total daily fluoride intake among the social areas, genders and age groups in each collection.

8.6. Conclusion

From the findings of this chapter it can be concluded that:

- Dietary fluoride intake of children estimated by each method was 0.03 mg/kg bw /d which was below the "so called" range of 0.05-0.07 mg/kg bw/d.
- Total daily dietary fluoride intakes obtained from each dietary assessment method was within the optimal range at the group level
- On individual basis total daily fluoride intake of more than half of the children in the present study was below the optimal range.
- The contribution of toothpaste to total daily fluoride intake in both collections was found to be almost equal to the contribution of diet.
- Water by itself was not the major source of dietary fluoride intake in some children. However, when water was used for the preparation of food and drinks, it had a major impact on dietary fluoride intake for children in the present study.

Chapter 9 Urinary fluoride excretion, fractional urinary fluoride excretion and fluoride retention of children

9.1. Introduction

In this chapter the results of 24-h urinary fluoride excretion are presented with a discussion to relate these findings to the normative values suggested by the WHO (1999) for monitoring fluoride exposure of communities.

9.2. Aims

The aims of this chapter were to:

- estimate urinary fluoride excretion
- compare the excretion data with the WHO guidelines

The objectives were to estimate:

- fractional urinary fluoride excretion
- fractional fluoride retention

9.3. Materials and methods

Methods of 24-h urine collections were presented in Chapter 5, section 5.2.4.
In summary one 24-h urine sample was collected along with each dietary assessment method.

9.4. Results

The results of urinary fluoride excretion for each collection are presented separately.

9.4.1. Urinary fluoride excretion (2-day duplicate method)

9.4.1.1 Urine volumes

Mean (SD) daily urine volumes was 516 (271) ml with a range from 165 to 1301 ml/d. Duration of daily collection of urine for all children ranged from 21h 41min to 30h 24min with a mean of 23h 54 min.
The volume of urines was corrected for the 24-h as described in Chapter 5, section 5.3.1.
Table 9.1 presents corrected urine volumes for 24-h and on a body weight basis.

Mean corrected urine volumes in boys (534 ml/d) and girls (492ml/d) were fairly close to each other. On body weight basis the mean corrected urine volumes (ml) were almost identical in all age groups. The point estimate of the mean corrected urine volumes in children from the high social area was slightly higher (531 ml/d) than that of in children from the low social area (507 ml/d).

Table 9.1 Mean (SD) and range of corrected urine volumes by gender, age and social area [number of children]

	Corrected urine volumes			
	ml/d		ml/kgbw/d	
	Mean (SD)	Range	Mean	Range
Gender:				
- Boys [n=35]	534 (284)	165-1301	25 (13)	7-56
- Girls [n=26]	492 (257)	201-1174	24 (11)	10-55
Age (yrs)				
- 4 [n=20]	506 (323)	169-1301	26 (13)	9-55
- 5 [n=22]	472 (283)	165-1174	23 (13)	7-56
- 6 [n=19]	578 (190)	336-930	25 (8)	15-41
Social area				
- LSE [n=38]	507 (255)	165-1059	24 (12)	7-56
- HSE [n=23]	531 (303)	242-1301	25 (12)	11-55
All [n=61]	516 (272)	165-1301	24 (12)	7-56

9.4.1.2. Daily Urinary Fluoride Excretion (DUFE)

Table 9.2 presents mean (SD) daily urinary fluoride excretion per day and per kg body weight. Mean (SD) daily urinary fluoride excretion for all children was 0.368 (0.169).mg /d and 0.018 (0.008) mg/kg bw/d when expressed on body weight basis.

According to the results, mean daily urinary fluoride excretion on body weight basis was almost identical between genders, social areas, and age groups.

Table 9.2 Mean (SD) and range of DUFE for children according to gender, age and social area [number of children]

	Daily urinary fluoride excretion			
	mg/d		mg/kg bw/d	
	Mean (SD)	Range	Mean (SD)	Range
Gender:				
- Boys [n=35]	0.393 (0.191)	0.117-0.790	0.019 (0.010)	0.005-0.041
- Girls [n=26]	0.334 (0.131)	0.096-0.556	0.016 (0.006)	0.005-0.031
Age (yrs)				
- 4 [n=20]	0.334 (0.188)	0.096-0.714	0.018 (0.011)	0.005-0.041
- 5 [n=22]	0.364 (0.161)	0.135-0.790	0.018 (0.008)	0.006-0.039
- 6 [n=19]	0.408 (0.158)	0.171-0.789	0.017 (0.007)	0.007-0.036
Social area				
- LSE [n=38]	0.376 (0.190)	0.117-0.790	0.018 (0.009)	0.005-0.041
- HSE [n=23]	0.355 (0.131)	0.096-0.582	0.017 (0.007)	0.005-0.031
All [n=61]	0.368 (0.169)	0.096-0.790	0.018 (0.008)	0.005-0.041

9.4.1.3. Fractional Urinary Fluoride Excretion (FUFE)

The fractional urinary fluoride excretion ranged from 7% to 106% with a mean of 37% for all children (Table 9.3). FUFE of boys was slightly higher (39%) with a range from 7% to 106%.

Among the three age groups, a higher fraction of fluoride was excreted in urine by 6 year olds (42%) compared with 4 and 5 year old children.

Table 9.3 Fractional urinary fluoride excretion (%) for all children [number of children]

	FUFE (%)	
	Mean (SD)	Range
Gender:		
- Boys [n=35]	39 (25)	7-106
- Girls [n=26]	34 (16)	10-73
Age (yrs)		
- 4 [n=20]	38(28)	10-106
- 5 [n=22]	33(16)	7-67
- 6 [n=19]	42 (21)	19-80
Social area		
- LSE [n=38]	37 (25)	7-106
- HSE [n=23]	38 (15)	16-71
All [n=61]	37 (22)	7-106

9.4.1.4. Daily Fluoride Retention (DFR) and Fractional Fluoride Retention (FFR)

Mean daily fluoride retention and fractional fluoride retention of all children are presented in Table 9.4. Mean (SD) daily fluoride retention for all children was 0.740 (0.700) mg/d and 0.036 (0.037) mg/kg bw/d. Daily fluoride retention on body weight basis was similar in boys and girls (0.036 mg/kgbw/d).

On the basis of body weight, daily fluoride retention decreased by age.

Fractional fluoride retention for all children was 53% with a range from -16% to +83%.

The point estimate of fractional fluoride retention was higher in 5 year olds compared with the other age groups.

Table 9.4 Daily fluoride retention and fractional fluoride retention of all children and by gender, age and social area (collected along with 2-day duplicate method)

	Daily F retention					
	mg/d		mg/kg bw/d		Fractional F retention (%)	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Gender:						
- Boys [n=35]	0.779 (0.788)	-0.108,+4.205	0.036 (0.038)	-0.006,+0.208	51 (25)	-16,+83
- Girls [n=26]	0.688 (0.571)	0.110, +2.340	0.036 (0.036)	0.004,+0.135	55 (16)	17,+80
Age (yrs)						
- 4 [n=20]	0.768 (0.744)	-0.108,+2.340	0.042 (0.044)	-0.006,+0.135	52 (27)	-16,+80
- 5 [n=22]	0.842 (0.852)	0.199-4.205	0.041 (0.042)	0.009,+0.208	57 (16)	23,+83
- 6 [n=19]	0.592 (0.405)	0.057,+1.358	0.025 (0.017)	0.002,+0.054	48 (20)	10,+71
Social area						
- LSE [n=38]	0. 822 (0.788)	-0.108,+4.205	0.046 (0.045)	-0.002,+0.236	53 (25)	-16,+83
- HSE [n=23]	0.604 (0.511)	0.125,+2.340	0.036 (0.035)	0.009,+0.148	52 (15)	18,+73
All (n=61)	0.740 (0.700)	-0.108,+4.205	0.036 (0.037)	-0.006,+0.208	53 (22)	-16,+83

9.4.1.5. Correlation between fluoride intake and excretion

Table 9.5 presents total daily fluoride intake and excretion, total daily fluoride retention, fractional fluoride retention and excretion for all children.

Table 9.5 Summary of F intake and excretion data for all children [n=61]

	Mean (SD) [n=61]
Total daily fluoride intake	
- mg/d	1.231 (0.839)
- mg/kg bw/d	0.060 (0.045)
Daily urinary fluoride excretion	
- mg/d	0.368 (0.169)
- mg/kg bw/d	0.018 (0.008)
Fractional urinary fluoride excretion (%)	37 (22)
Daily fluoride retention (mg/d)	0.740 (0.700)
Fractional fluoride retention (%)	53 (22)

The correlation between total daily fluoride intake and:

i. urinary fluoride excretion was moderate, positive and statistically significant ($r=0.43$, $p<0.001$) (Figure 9.1).
ii. fractional urinary fluoride excretion (%) was moderate, negative, and statistically significant ($r=-0.47$, $p<0.001$) (Figure 9.2).
iii. total daily fluoride retention (mg/d) was strong positive and statistically significant ($r=0.98$, $p<0.001$) (Figure 9.3).
iv. fractional fluoride retention (%) was also moderate, positive and statistically significant ($r=0.51$, $p<0.001$) (Figure 9.4).

Figure 9.1 Correlation between total daily fluoride intake (mg/d) and daily urinary fluoride excretion (mg/d) for all children (n=61)

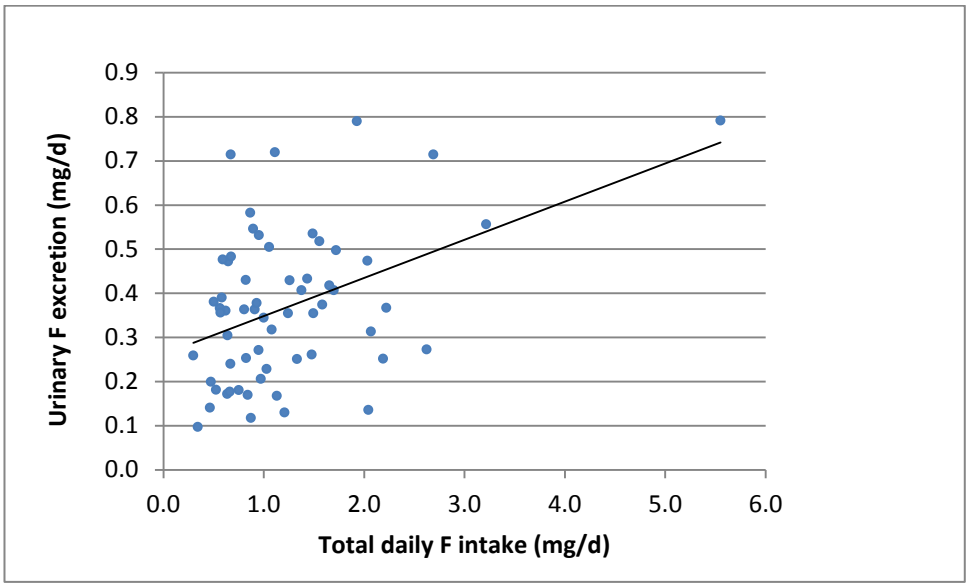


Figure 9.2 Scatter plot between total daily F intake (mg/d) and FUFE (%) (n=61)

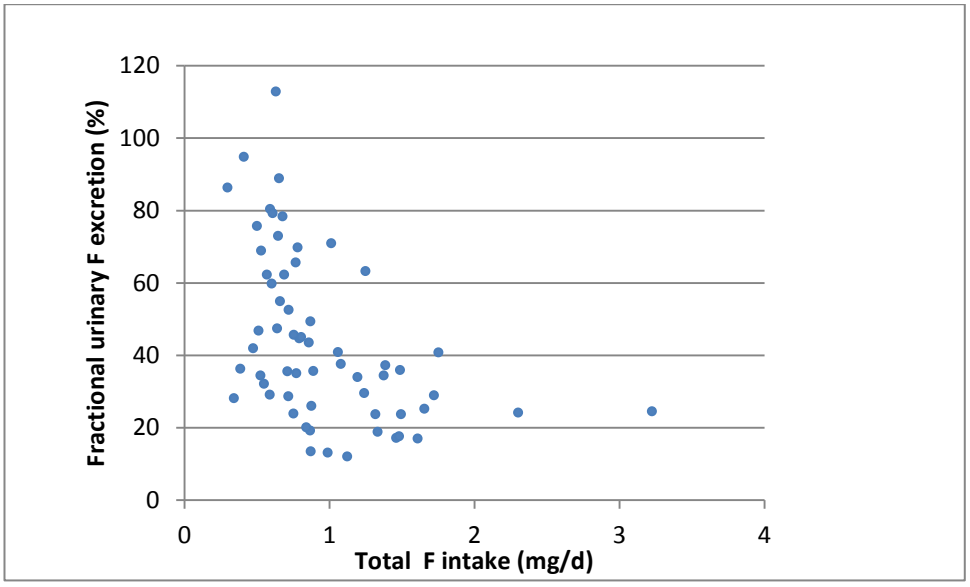


Figure 9.3 Correlation between total F intake (mg/d) and F retention (mg/d) (n=61)

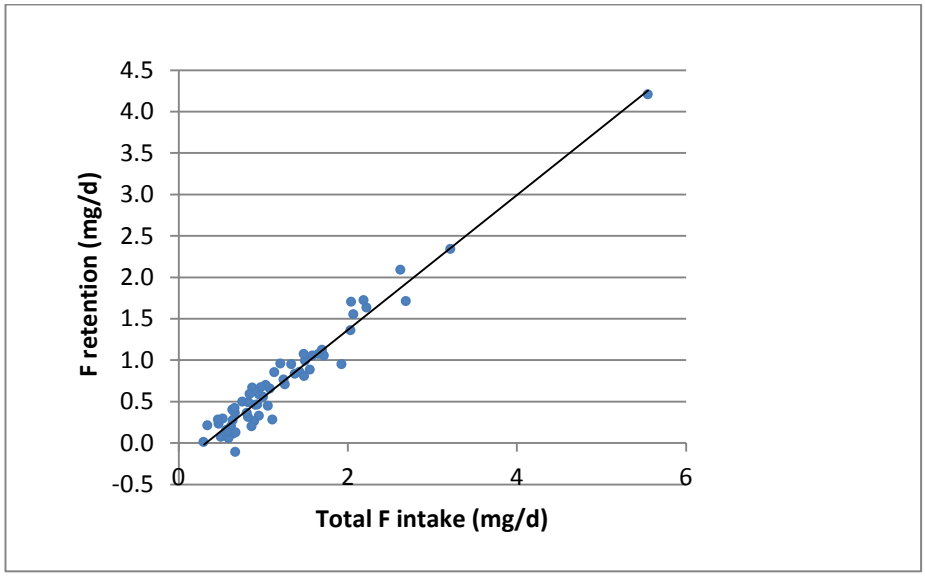
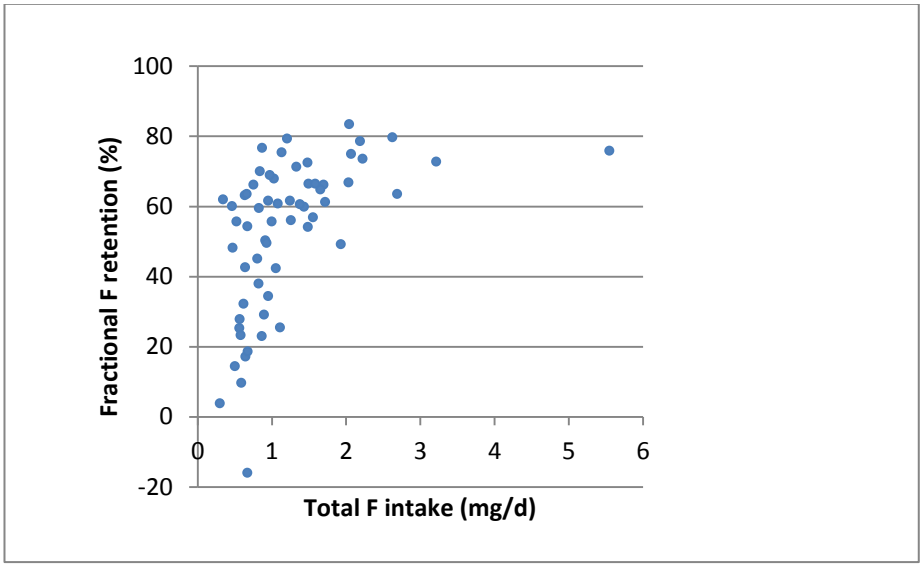


Figure 9.4 Scatter plot between total daily F intake (mg/d) and FFR (%)



9.4.2. Urinary fluoride excretion (3-day food diary method)

9.4.2.1. Urine volumes

The mean (SD) urine volumes was 482 (179) ml with a range from 148-1014 ml/d. Duration of daily collection of urine for all children ranged from 21h 5min to 27h 30 min with a mean of 23h 54 min.

Table 9.6 presents corrected urine volumes for the 24 hour period and on a body weight basis.

The mean corrected urine volumes in boys (473 ml/d) and girls (494 ml/d) were fairly close. The mean corrected 24h urine volumes were also similar in the three age groups, particularly when they were expressed on a body weight basis: 24, 23 and 22 ml/kg bw/d in 4, 5 and 6 year olds, respectively.

The point estimate of the mean corrected urine volume (ml) for children from the high social area was slightly higher (531 ml/d) than that in children from the low social area (453 ml/d).

Table 9.6 Mean (SD) and range of corrected urine volumes for all children and by gender, age and social area [number of children]

	Corrected urine volumes			
	ml/d		ml/kgbw/d	
	Mean (SD)	Range	Mean (SD) Range	
Gender:				
- Boys [n=35]	473 (161)	148-800	24 (9)	7-53
- Girls [n=26]	494 (203)	163-1014	22 (8)	5-39
Age (yrs)				
- 4 [n=20]	472 (170)	188-956	24(7)	11-39
- 5 [n=22]	496 (194)	148-1014	24 (8)	7-41
- 6 [n=19]	477 (178)	163-791	22(10)	5-53
Social area				
- LSE [n=38]	453 (163)	148-800	22 (8)	5-53
- HSE [n=23]	531 (196)	264-1014	24 (8)	7-39
All [n=61]	482(179)	148-1014	23 (8)	5-53

9.4.2.2. Daily Urinary Fluoride Excretion (DUFE)

Mean (SD) daily urinary fluoride excretion per day as well as on body weight basis for all children by gender, age and social area are presented in Table 9.7.

Overall mean (SD) daily urinary fluoride excretion for all children was 0.373 (0.205) mg/d and 0.018 (0.009) mg per kg body weight.

According to the results, mean daily urinary fluoride excretion when expressed on body weight basis was similar between genders, age groups, and social areas.

Table 9.7 Mean (SD) and range of daily urinary fluoride excretion for children according to gender, age and social area

	Daily urinary fluoride excretion			
	mg/d		mg/kgbw/d	
	Mean (SD)	Range	Mean(SD)	Range
Gender:				
- Boys [n=35]	0.385 (0.223)	0.133-1,029	0.018 (0.009)	0.009-0.043
- Girls [n=26]	0.357 (0.182)	0.076-0.765	0.018 (0.009)	0.003-0.039
Age (yrs)				
- 4 [n=20]	0.333 (0.158)	0.129-0.679	0.018 (0.009)	0.007-0.039
- 5 [n=22]	0.398 (0.170)	0.156-0.795	0.019 (0.008)	0.008-0.039
- 6 [n=19]	0.387 (0.278)	0.076-1.029	0.016 (0.010)	0.003-0.043
Social area				
- LSE [n=38]	0.371 (0.234)	0.076-1.029	0.017 (0.010)	0.003-0.043
- HSE [n=23]	0.377 (0.149)	0.129-0.795	0.018 (0.008)	0.007-0.039
All [n=61]	0.373 (0.205)	0.076-1.029	0.018 (0.009)	0.003-0.043

9.4.2.3. Fractional Urinary Fluoride Excretion (FUFE)

The fractional urinary fluoride excretion ranged from 6-189% with a mean of 41% for all children (Table 9.8).

As presented in Table 9.8, fractional urinary fluoride excretion was similar between genders and the social areas. The results showed a lower fractional urinary fluoride excretion for 6-year olds (31%) compared with the younger age groups.

Table 9.8 Mean fractional urinary fluoride (%) excretion for all children

	FUFE (%)	
	Mean (SD)	Range
Gender:		
- Boys [n=35]	41(30)	6-189
- Girls [n=26]	40 (20)	9-113
Age (yrs)		
- 4 [n=20]	46 (35)	17-189
- 5 [n=22]	44 (23)	6-113
- 6 [n=19]	31 (13)	9-52
Social class		
- LSE [n=38]	38 (30)	6-189
- HSE [n=23]	45 (18)	27-113
All [n=61]	41 (26)	6-189

9.4.2.4. Daily Fluoride Retention (DFR) and Fractional Fluoride Retention (FFR)

Mean daily fluoride retention and fractional fluoride retention of all children are presented in Table 9.9.

Mean (SD) daily fluoride retention of all children was 0.618 (0.521) mg/d and 0.029 (0.025) mg/kgbw/d. Daily fluoride retention based on body weight was similar in boys (0.031 mg) and girls (0.027 mg). Fluoride retention per day increased by age. However, on the body weight basis daily fluoride retention was similar among the three age groups as well as the two social areas and both genders.

Overall, fractional fluoride retention for all children was 49% with a wide range from -99% to +84%. The fractional fluoride retention was higher in 6 year olds (59%) compared with younger age groups whereas between boys and girls it was similar.

Table 9.9 Daily fluoride retention and fractional fluoride retention of all children by gender, age and social area (collected along with the 3-day food diary method)

	Daily F retention				Fractional F retention (%)	
	mg/d		mg/kgbw/d			
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Gender:						
- Boys [n=35]	0.659 (0.575)	-0.235,+2.807	0.031 (0.027)	-0.013, +0.139	49 (30)	-99,+84
- Girls [n=26]	0.562 (0.443)	-0.100,+1.835	0.027 (0.020)	-0.005,+0.072	50 (20)	-23,+81
Age (yrs)						
- 4 [n=20]	0.476 (0.408)	-0.235,+1.305	0.025 (0.021)	-0.013, +0.070	44 (35)	-99,+73
- 5 [n=22]	0.609 (0.615)	-0.100,+2.807	0.030 (0.031)	-0.005,+0.139	46 (23)	-23, +84
- 6 [n=19]	0.776 (0.490)	0.317, +1.870	0.032 (0.020)	0.012,+0.083	59 (13)	38, +81
Social area						
- LSE [n=38]	0.708 (0.600)	-0.235,+2.807	0.033 (0.028)	-0.013,+0.139	52 (29)	-99, +84
- HSE [n=23]	0.468 (0.311)	-0.100,+1.263	0.023 (0.017)	-0.005, +0.070	45 (18)	-23, +63
All [n=61]	0.618 (0.521)	-0.235, +2.807	0.029 (0.025)	-0.013, +0.139	49 (26)	-99, +84

9.4.2.5. Correlations between fluoride intake and excretion

Table 9.10 presents total daily fluoride intake, total daily fluoride excretion, fractional urinary fluoride excretion, daily fluoride retention and fractional fluoride retention for 61 children.

Table 9.10 Summary of F intake and excretion data for all children [n=61]

	Mean (SD) [n=61]
Total daily fluoride intake: - mg/d - mg/kgbw/d	 1.101 (0.663) 0.052 (0.031)
Daily urinary fluoride excretion: - mg/d - mg/kgbw/d	 0.373 (0.205) 0.018 (0.009)
Fractional urinary fluoride excretion (%)	41 (26)
Daily fluoride retention (mg/d)	0.618 (0.521)
Fractional fluoride retention (%)	49 (26)

Correlation between total daily fluoride intake and

i. Urinary fluoride excretion was moderate, positive and statistically significant ($r=0.45$, $p<0.001$) (Figure 9.5).
ii. Fractional urinary fluoride excretion (%) was moderate, negative and statistically significant ($r=-0.42$, $p<0.001$) (Figure 9.6).
iii. Total daily fluoride retention (mg/d) was strong, positive and statistically significant ($r=0.94$, $p<0.0001$) (Figures 9.7).
iv. Fractional fluoride retention (%) was moderate, positive and statistically significant ($r=0.44$, $p<0.0001$) (Figure 9.8).

Figure 9.5 Correlation between total daily fluoride intake and daily urinary fluoride excretion (mg/d) for all children

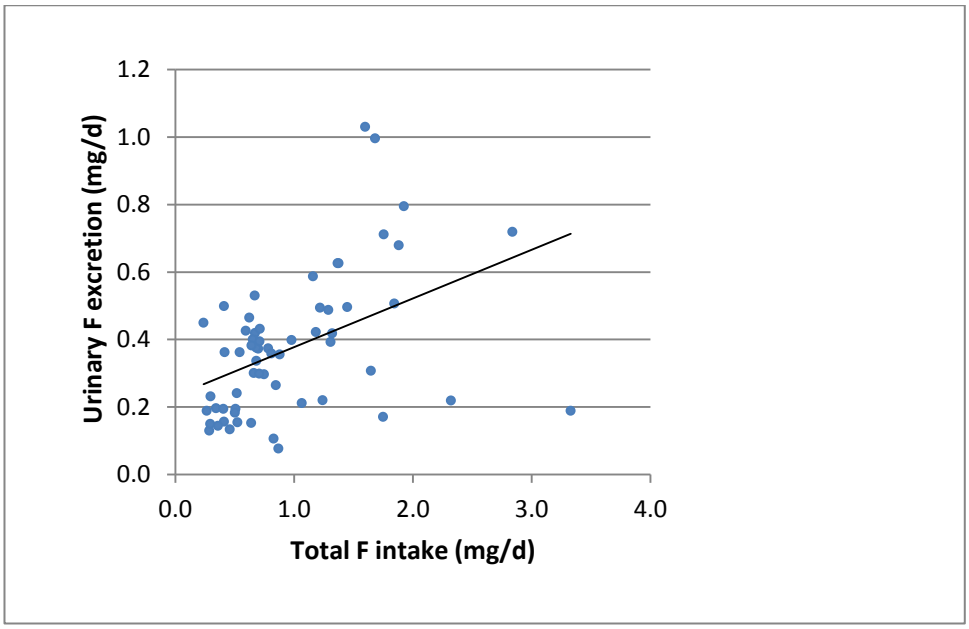


Figure 9.6 Scatter plot between total daily F intake (mg/d) and FUFE (%) [n=61]

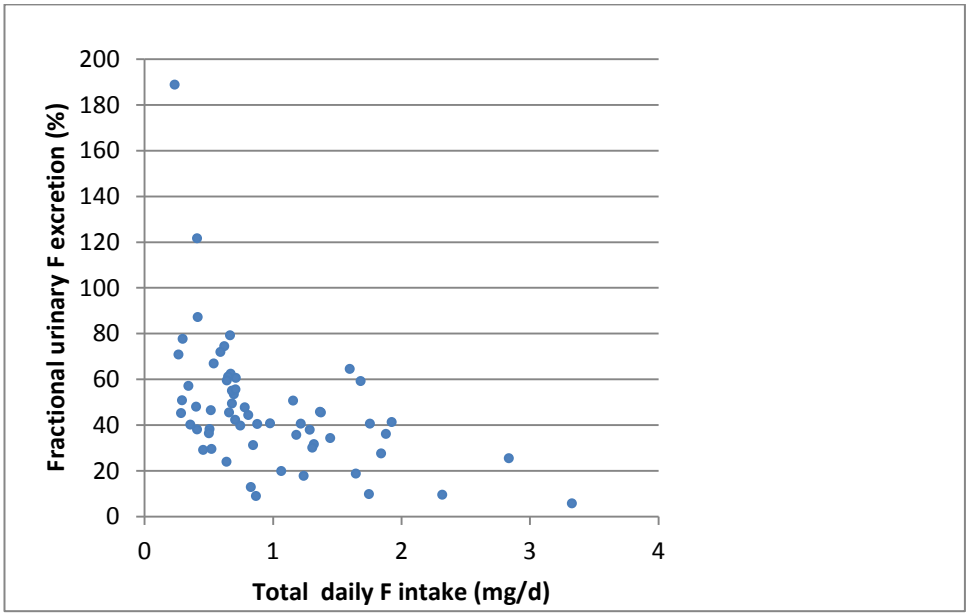


Figure 9.7 Correlation between total F intake (mg/d) and total F retention (mg/d) for all children

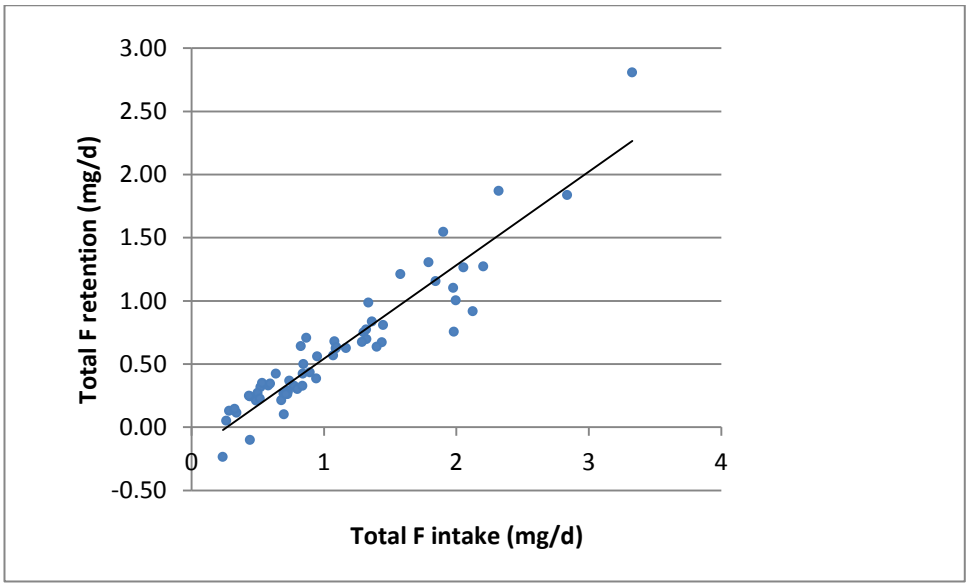
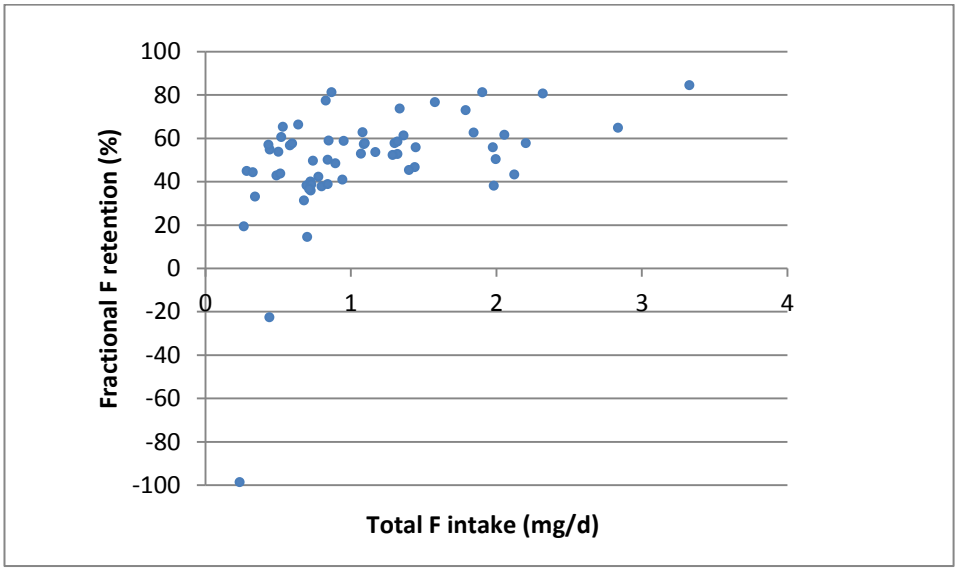


Figure 9.8 Scatter plot between total daily F intake (mg/d) and FFR (%) for all children



9.5 Discussion

9.5.1. Urine volumes

The correction of urine volumes for 24-h has been recommended and described by the WHO (Marthaler 1999). In this study the duration of urine collection varied for each child. It was therefore decided to correct the urine volumes for 24-h in order to have the same duration for the whole study population. Urine volumes have previously been corrected for 24-h by Ketley et al. in a UK study which determined urinary fluoride excretion of children drinking fluoridated milk (Ketley & Lennon 2000). The mean corrected 24-h urine volume for children in this study was 516 ml/d for one collection. This was slightly higher than the corrected urine volumes of 1.5-3.5 year old children in a European study (Ketley *et al.,* 2004). Urine volumes in Ketley's study reported to be 499 ml/d for children in Cork (Ireland), 498ml/d for children in Knowsley (England) and 440 ml/d for children in Reykjavik (Iceland). In a study of older children living in a fluoridated area of the north-east of England, 24-h urine volumes were reported to be 449 ml/d for 4 year old (Rugg-Gunn *et al.,* 1993) and 495 ml/d for 6 year old children (Maguire *et al.,* 2007). However, the mean urine volume of 482 ml/d which was obtained from another collection (along with 3-day food diary method) in the present study was found to be in agreement with the above ranges. Overall, the mean urine volumes form both collections in the present study were lower than the corresponding values of 568 ml/d reported for 3-5 year olds German children (Haftenberger *et al.,* 2001), 646, 540 and 726 ml/d reported for 1.5-3.5 year old children from Oulu (Finland), Haarlem (Netherlands) and Almada/Setubal (Portugal) respectively (Ketley *et al.,* 2004).

Difference in the urine volumes could be related to the differences in the amount of fluid intake.

9.5.2. Fluoride excretion

Mean 24-h urinary fluoride excretion of children in the present study at 0.370 and 0.373 mg/d were close to the reported values of 0.370 mg/d for 3 year old children from Ireland (Ketley *et al.,* 2004), 0.358 mg/d for 3.5 year old Chilean children (Villa *et al.,* 2000) and 0.323 mg/d for 6-7 year old British children (Maguire *et al.,* 2007) respectively. However, on body weight basis the mean urinary fluoride

excretion of 0.018 for children in the present study was higher than 0.014 mg/kg bw/d reported for British children (Maguire *et al.*, 2007) while it was slightly lower than the value of 0.022 mg/kg bw/d reported for 3 year old children from Ireland (Ketley *et al.*, 2004) and 3-5 year old children from Chile (Villa *et al.*, 2000). This discrepancy could be partly explained by age as British children were older while children from Ireland and Chile were younger than the children in the present study.

The mean urinary fluoride excretion in the present study for both collections was lower than 0.420, 0.550 and 0.476 mg/d reported for pre-school children living in optimally fluoridated areas in England and Sri Lanka (Rugg-Gunn *et al.*, 1993) and Germany (Haftenberger *et al.*, 2001) respectively. A lower excretion of 0.245, was found in Swiss children aged 3-4 years consuming fluoridated salt (Marthaler *et al.*, 2000). Several reasons may contribute to the differences in the rate of urinary fluoride excretion among children living in optimally fluoridated areas. Urine pH and flow rate are the factors which affect the renal clearance of fluoride (Ekstrand *et al.*, 1988). When the pH of tubular fluid is low (acidic), more fluoride ions are converted to Hydrogen Fluoride (HF) which is diffused across the tubular epithelium. On the contrary, when the tubular fluid is alkaline (pH is high), nearly all of the fluoride exists in the ionic form and remains within the tubule which is excreted (Whitford 1990). Diet composition is another factor contributes to the difference in the rate of fluoride excretion. A vegetarian rich diet could increase the urine pH and therefore would be associated with the greater fluoride excretion. The altitude of residence also influences urine pH. Residency in high altitudes would reduce the urine pH and consequently renal excretion of fluoride. In addition to diet and altitude, individual variation in fluoride metabolism contribute to the different rates of fluoride excretion.

A rate of provisional standards for the urinary fluoride excretion has been proposed by the WHO (Marthaler 1999) for 3-7 year old children living in optimally fluoridated areas which are between 0.360 to 0.600 mg/d. These provisional standards can be used as a measure of fluoride exposure of the community. The rate of 0.370 mg/d for children in this study at the group level was found to be within the range proposed by the WHO. However, the use of WHO standards has a limitation to the extent that the ranges have been established on the basis of small number of studies which are quite old. Besides,

these standards have not been established on body weight basis, while the "so-called" optimal intake is based on body weight.

9.5.3. Correlation between fluoride intake and excretion

The correlation between intake and excretion of fluoride in the present study was positive and statistically significant. The relationship between fluoride intake and excretion parameters are discussed in the following sub-sections.

9.5.3.1. Fractional Urinary Fluoride Excretion (FUFE)

The high cost and time involved in measuring total fluoride intake from diet as well as varying degree of gastrointestinal fluoride absorption from different sources of fluoride intake such as diet and its components and dental care products, may limit the value of estimated fluoride exposure with regards to the systematic effect of fluoride. Given these limitations, the fraction of fluoride eliminated with urine can be used for estimating fluoride intake (Haftenberger *et al.*, 2001). However, a relatively constant urinary excretion rate in an age group or growing period has not been established. Hence, the most accurate means of determining fractional urinary fluoride excretion could be by direct measurement of fluoride content of all ingested food, beverages and toothpaste in addition to collection of 24-h urinary fluoride excretion data. A limited number of studies have reported both intake and excretion and these studies have been conducted in different age groups 3-5, 4 and 6-7 year olds (Villa *et al.*, 2000, Zohouri & Rugg-Gunn 2000b, Maguire *et al.*, 2007).

While the proportion of ingested fluoride that is excreted in urine is about 50% in adults, in children this percentage suggested to be lower at 30%-40% (Murray 1986b, Ekstrand & Whitford 1988,). The proportion of total daily fluoride intake excreted in the urine in pre-school children has been estimated by few studies and their results are summarised in Table 9.11 (Brunetti & Newbrun 1983, Ekstrand *et al.*, 1994, Villa *et al.*, 1999, Ketley & Lennon 2000, Villa *et al.*, 2000, Zohouri & Rugg-Gunn 2000b, Haftenberger *et al.*, 2001, Franco *et al.*, 2005, Maguire *et al.*, 2007).

The reported fractional urinary fluoride excretion in pre-school children ranged from 30% in 4-5 year old English (Ketley & Lennon 2000) to 85% in 3-4 year old US children (Brunetti & Newbrun 1983). The mean fractional urinary fluoride

excretion of 37% obtained for one collection in the present study was fairly close to 32% reported for 6-7 year old British children (Maguire *et al.,* 2007) living in the same locality as children in the current study, 35% for 3-5 year old Chilean children (Villa *et al.,* 2000) and 33% for 4-5 year old Colombian children (Franco *et al.,* 2005b). However, higher value of 41% was obtained from another collection (collection along with 3-day food diary method) in this study which was similar to 43% reported for formula-fed infants (Ekstrand 1994). Higher fractional urinary fluoride excretions of 52% and 80% have been reported for 3-6 year old German (Haftenberger *et al.,* 2001) and 4 year old Iranian children (Zohouri & Rugg-Gunn 2000b) respectively. One of the factors reported to be associated with high excretion rate in Iranian children was diet composition which was mainly vegetarian (Zohouri & Rugg-Gunn 2000b).

Table 9.11 Summary of studies reported Fractional Urinary Fluoride Excretion

Author, year, country	**Age (number of children)**	**FUFE%**
Brunetti & Newbrun 1983, USA	3-4 (10)	85
Ekstrand et al., 1994, USA	0.19-0.89$^{\sigma}$(11)	78
Ekstrand et al., 1994, USA	0.18-0.93$^{\delta}$(20)	43
Villa et al., 1999, Chile	3-5 (48)	31
Villa et al., 2000, Chile	3-5 (20)	35
Ketley & Lennon, 2000, UK	4-5 (8)	30
Zohoori et al., 2000, Iran*	4 (78)	80
Haftenberger et al., 2001, Germany*	3-6 (11)	52
Franco et al., 2005, Colombia	4-5 (96)	33
Maguire et al., 2007, UK	6-7 (6)	32
Present study, 2010,UK	4-6 (61)	37

*low fluoride in drinking water (0.3mgF/l), $^{\sigma}$ Formula fed, $^{\delta}$ Formula-fed with F supplement

9.5.3.2. Daily fluoride retention and fractional fluoride retention

To report fluoride retention for children in the present study, the faecal excretion of fluoride was added to the urine excretion. The faecal excretion was not experimentally determined and was based on the study by Ekstrand (Ekstrand *et al.,* 1984) who found that almost 10% of daily ingested fluoride was excreted through faeces in infants aged 8-28 weeks living in an optimally fluoridated area.

Daily fractional fluoride retention of 49% for children in this study was slightly lower than daily fluoride retention of 54% and 58% reported for 3-5 year old Chilean (Villa *et al.,* 2000) and 6-7 year old British children (Maguire *et al.,* 2007). However, the value of 53% obtained from another collection in this study was consistent with the reported values by the above studies.

Lower fluoride retention at 12.5%, 11%, and 15% has been reported for formula-fed infants (Ekstrand 1994), and 4 year old Iranian (Zohouri & Rugg-Gunn 2000b) and North American children (Brunetti & Newbrun 1983).

In this study two children were in negative fluoride balance. While the fraction of ingested fluoride excreted in the urine for one child was high in both collections (106% and 188%) this fraction was high for another child only at one collection (112%). Negative balances have been reported in breast-fed infants with fluoride intake of 5 to 19 µg/d (Ekstrand *et al.,* 1984) and less than 500µg/d in adults (Maheshwari *et al.,* 1981). On the other hand positive balances have been reported by these studies for formula-fed infants ingesting fluoride from 891 to 1012 µg/d and adults ingesting 5000 to 10000 µg fluoride per day.

Negative balances can be explained by the differences in the rate of fluoride uptake into bones and teeth which depends on the stage of skeletal development of children and uptake of fluoride is quicker in newly formed bones than in the mature bones (Whitford 1994). Consequently, during periods of rapid growth, fluoride retention is greater. The periods of negative balance may occur most often when fluoride intake is reduced which results in the reduction of plasma concentration of fluoride. The rate of fluoride uptake is proportional to the plasma fluoride concentration. At the lower intake of fluoride the ion is mobilised from calcified tissues and becomes available for urinary excretion (Whitford 1994).

Another explanation for negative balances in children is the difference in the diet composition. Insoluble complex formation of calcium and magnesium with fluoride in the diet will reduce fluoride absorption and its uptake into teeth and bone. While, a diet rich in protein and fat increases the absorption and results in an increase in the proportion of fluoride intake retained in the body. (Cerklewski 1997).

When the proportion of total daily fluoride intake which was retained by the children in this study was examined, this proportion for the majority of children increased sharply at a total daily fluoride intake of up to 1 mg/d (Figures 9.4 and

8). In a recent study the relationship between total daily fluoride intake and urinary fluoride excretion was examined using previously published data on fluoride intake and urinary fluoride excretion in adults and children (Villa et al., 2010). In that study Villa et al. suggested that for total daily fluoride intake of almost >0.5 mg/d the fractional fluoride retention could reach to limiting constant value irrespective of how high was the total daily fluoride intake. The 1 mg total daily fluoride intake found in the present study was as twice as what was suggested by Villa and co-workers to reach constant values in the fractional fluoride retention. However, this difference can be attributed to the narrow age range (4-6 years) in the current study compared to 6 months to 7 years old age range for the study by Villa et al.

9.5.4. Urinary fluoride excretion and fluoride retention between social areas, genders and age groups (both collections)

As described in chapter 8, due to the sample size calculation to address the aim of the study, the statistical analysis could not be carried out on fluoride excretion variables between social areas, genders and the age groups. However, the descriptive data showed that on body weight basis no mathematically considerable differences was observed in urinary fluoride excretion and fluoride retention between the social areas, genders and the age groups.

9.6. Conclusion

From the results of this study it can be concluded that:

- Mean urinary fluoride excretion of children in both collections was within the provisional standards of 0.360-0.600 mg/d recommended by the WHO (Marthaler 1999).
- The wide range of urinary fluoride excretion found in both collections (from 0.096 to 0.790 mg/d) suggested that on individual basis most of children did not receive the optimal fluoride level. While some of the children received fluoride above the optimal range.
- Mean fractional urinary fluoride excretion of children in the present study were 37% and 41%.
- Fractional fluoride retention in both collections showed that except for two children, the rest of children were in positive fluoride balance.

Chapter 10 Comparison between dietary assessment methods and collections of urines and expectorated saliva/toothpaste/rinse

10.1. Introduction

This chapter provides a comparative analysis between dietary assessment methods in estimating fluoride intake at the group and individual level. Daily variation in fluoride intake from diet and toothpaste and variation in urinary fluoride excretion have also been investigated and discussed in this chapter.

10.2. Aim

The main aim of this part of the study was to compare dietary fluoride intake measured using "2-day duplicate" and "3-day food diary" methods at the group and individual level.

The objectives of the study were to investigate daily variation in: i) dietary fluoride intake ii) fluoride intake from toothpaste ingestion and iii) urinary fluoride excretion at both group and individual levels.

10.3. Materials and methods

Methods of sample and data collection have been previously explained in details in Chapter 5. This chapter mainly describes the statistical analysis of the collected data for the purpose of comparison at the group and individual level.

10.3.1. Statistical analysis

A paired t-test was conducted to compare the 2-day duplicate and 3-day food diary methods for the following variables at the group level:

- weight of consumed food, drinks and water
- dietary fluoride intake as well as total fluoride intake (toothpaste and diet)
- the contribution of the main 3 dietary source to total daily fluoride intake

Dietary data at the individual level were compared using Bland-Altman limits of agreement and associated plot (Bland & Altman 1986). Agreement between the two dietary methods was quantified using the differences between estimated dietary fluoride intakes by the two methods for the same subject. The 95% limits of agreement were then estimated by "mean difference ± 1.96 standard deviation of the difference". The difference in dietary fluoride intake between the 3-day

food diary and 2-day duplicate methods (y axis) was plotted against the mean fluoride intake averaged from the two methods (x-axis) for each child. Where there were substantial relationships between the difference and the mean, data were first log transformed and then back-transformed to give limits for ratio (Bland & Altman 1999).

The typical within-child error in dietary fluoride intake from one measurement (day) to another measurement was calculated by dividing SD of differences by √2.

10.4. Results

The results of this chapter are presented in 4 parts:

Part A: comparison between the two dietary assessment methods

Part B: variability in fluoride intake from toothpaste ingestion between collections

Part C: variability in urine volumes and urinary fluoride excretion between collections

Part D: summary data which is the average of dietary fluoride intake, fluoride intake from toothpaste and urinary fluoride excretion obtained from dietary assessment methods and two collections respectively.

10.4.1. Part A: Comparison between the two dietary assessment methods

- **Weight of consumed food, drinks, and water**

The mean weight of food, drinks, and water obtained by each method is presented in Table 10.1. There was no statistically significant difference in the mean weight of consumed drinks between the two methods. However, the mean weight of collected water by 2-day duplicate method was higher than the mean weight of reported water consumption in 3-day food diary and the difference was statistically significant ($p<0.001$). The mean weight of solid food reported by 3-day food diary was higher than the mean weight of that by 2-day duplicate method and the difference was statistically significant ($p<0.001$).

Table 10.1 Measured weight of food, drinks and water by 2-day duplicate and 3-day food diary methods [n=61]

Source	Weight (g) Mean (SD)			
	3-day food diary	2-day duplicate	Mean difference (95% CI)	P value
- Total drinks	432 (245)	533.(233)	-100 (-151, -48)	<0.001
- Water	139 (177)	204 (214)	-64 (-97, -32)	<0.001
- Other Drinks	293 (255)	329 (219)	-35 (-80, +9)	0.12
- Food	839 (233)	734 (193)	+105 (+50, +160)	<0.001

- **Estimated dietary fluoride intake by 2-day duplicate and 3-day food diary method**

Mean (SD) dietary fluoride intake from all dietary sources and total dietary fluoride intake estimated by each method is presented in Table 10.2.

There were no statistically significant differences in daily fluoride intake from drinks between the two dietary methods. The difference in fluoride intake from food between the two methods was also negligible. However, a higher fluoride intake from water was obtained by 2-day duplicate method which was statistically significant ($p<0.001$). No statistically significant difference was observed for the total dietary fluoride intake between the two methods per day and per kg body weight per day.

Table 10.2 Estimated dietary fluoride intake by methods [n=61]

Source	Dietary fluoride intake (mg/d) Mean (SD)		Mean difference (95% CI)	p value
	3-day food diary	2-day duplicate plate		
Total drinks	0.266 (0.218)	0.357 (0.221)	-0.091 (-0.140, -0.043)	<0.001
- Water	0.132 (0.173)	0.199 (0.205)	-0.067 (-0.099, -0.036)	<0.001
- Other drinks	0.134 (0.194)	0.158 (0.173)	-0.024 (-0.067,+0.019)	0.30
Food	0.267 (0.183)	0.225 (0.101)	+0.041(-0.001, +0.084,)	0.06
Total dietary F intake:				
- mg/d	0.533 (0.319)	0.583 (0.263)	-0.050 (-0.109, +0.009)	0.10
- mg/kg bw/d	0.025 (0.016)	0.028 (0.013)	-0.003 (-0.006, +0.000)	0.10

- **Contribution (%) of food and other drinks and water to daily dietary fluoride intake**

The contribution of each dietary source to total daily dietary fluoride intake by the two methods have been presented in Table 10.3. No significant difference was observed in the contribution of drinks to total daily fluoride intake between the two methods whereas statistically significant differences ($p<0.001$) were observed in the contributions of food and water to daily fluoride intake between the two methods.

Table 10.3 Contribution (%) of dietary sources to total daily fluoride intake by method

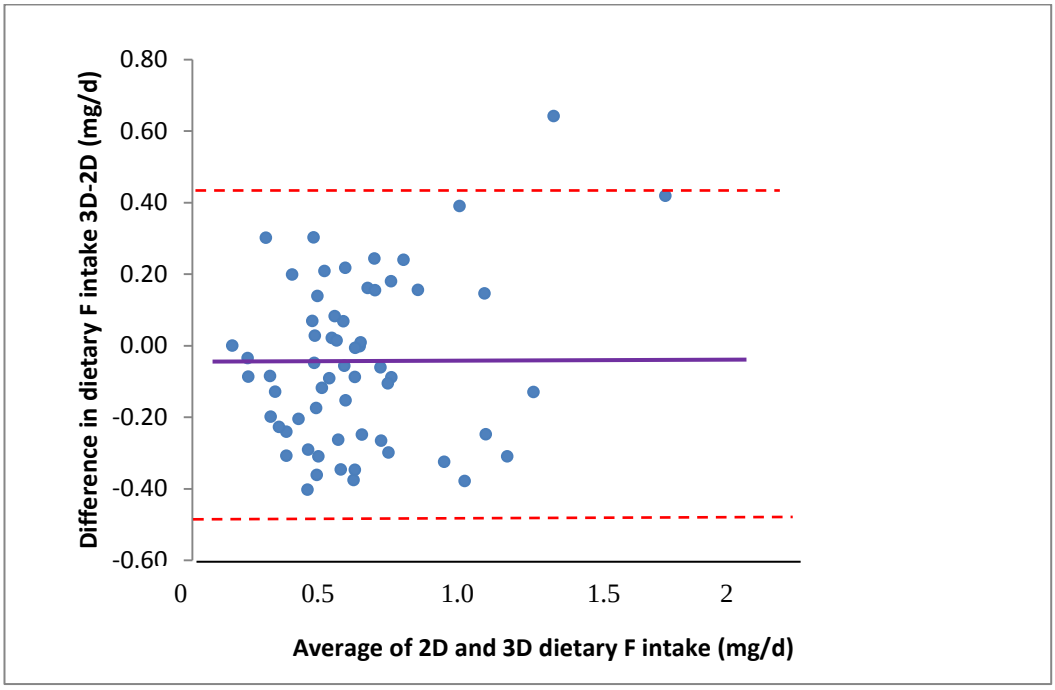
Source	Mean (SD) of contribution by method		Mean difference (95% CI)	P value
	3-day food diary	2-day duplicate		
Food	52 (20)	42 (17)	+9.6 (+4.1,+15.1)	<0.001
Total drinks	48 (20)	58 (17)	-9.6 (-15.1,-4.1)	<0.001
Other drinks	26 (24)	27 (23)	-0.6 (-6.1,+4.1)	0.83
Water	21 (20)	31 (22)	-9.0 (-14.1,-3.9)	<0.001

- **Agreement between dietary assessment methods for estimating dietary fluoride intake**

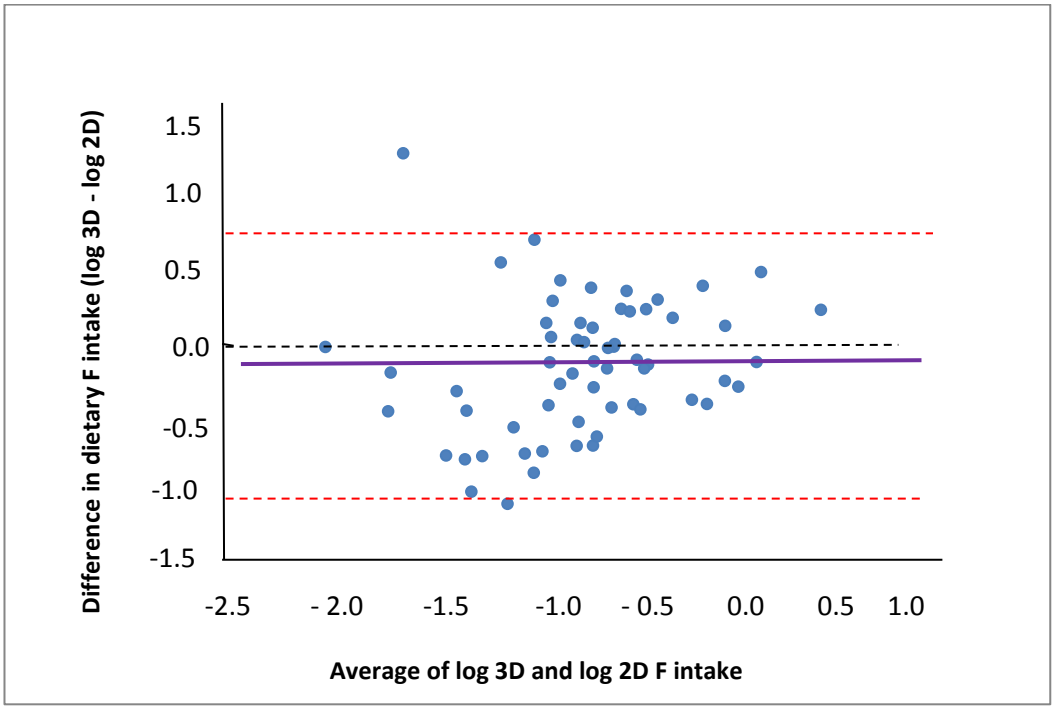
The mean (SD) difference in estimated dietary fluoride intake by the two dietary assessment methods for all 61 children was -0.050 (0.230) mg/d; with 95% limits of agreement of -0.501 to +0.401 mg/d (Figure 1a). However, there was a substantial relationship between the mean and the difference of dietary fluoride intake. Therefore, dietary data were log transformed. The mean difference was -0.144 with 95% limits of agreement of -1.08 to +0.8 on log scale. After taking the antilog of the log transformed data, the corresponding mean difference was 0.87 and the 95% limits of agreement were 0.87×/÷ 2.57 which indicated that the ratio of one method to the other was between 0.34 to 2.24.

Figure 10.1 Bland-Altman plot for 61 children: demonstrating the agreement between the two methods in estimating dietary F intake a) before log transformation, b) after log transformation

a)



b)



In these figures horizontal line (y=0) represents perfect agreement where the difference between methods are zero. The horizontal purple line indicates the mean of the differences (bias) which is -0.050 (Figure 1a) and -0.144 on a log scale (Figure 1b), the upper and lower red dashed lines show the upper and lower 95% limit of agreement respectively, presented as two fold SD (±1.96×SD).

The shift in the mean and the typical within-child variability (95% CI) in dietary fluoride intake from one measurement (day) to another measurement are presented in Table 10.4.

Table 10.4 Within child variability in daily fluoride intake estimated from each dietary method

Method	**Mean (SD) of daily dietary fluoride intake (mg/d)**			**Typical within-child variability (95% CI)**
	Day 1	Day 2	Day 3	
3-day food diary	0.555 (0.419)	0.508 (0.342)	0.536 (0.411)	±0.280 (0.240, 0.340)
2-day duplicate	0.574 (0.308)	0.592 (0.283)	-	±0.191 (0.160, 0.230)

10.4.2. Part B. Variability in fluoride intake from toothpaste ingestion between collections

Table 10.5 shows the results of fluoride intake from toothpaste ingestion between the two collections. No statistically significant difference was observed in the amount of toothpaste used and ingested per brushing and per kg body weight between the first and second collections.

The within child variation in toothpaste ingestion was ±0.254 mg/d (95% CI 0.300-0.440 mg/d). After log transformation of variables (fluoride ingestion per brushing) from both collections the within-child variability from test to re-test as a ratio of the mean was ×/÷ 2.07.

Table 10.5 Toothpaste usage and ingestion by children [n=61] at two collections

	Mean (SD)		Mean difference (95% CI)	P value
	2-day duplicate	3-day food diary		
Weight of TP used (g)	0.69 (0.50)	0.65 (0.34)	0.04 (-0.08, +0.16)	0.48
Weight of water used for rinsing (g)	68.7 (14.6)	65.9 (16.2)	2.70 (-1.66, +7.23)	0.21
F ingestion (mg) per: - brushing - day	 0.373 (0.395) 0.647 (0.762)	 0.339 (0.318) 0.568 (0.507)	 0.033 (-0.058, +0.125) 0.079 (-0.078, +0.237)	 0.46 0.31
F ingestion (mg/kg bw) per: - brushing - day	 0.018 (0.020) 0.032 (0.040)	 0.016 (0.013) 0.026 (0.023)	 0.002 (-0.002,+0.008) 0.005 (-0.002, +0.013)	 0.28 0.19
Swallowed F /brushing (%)	42 (19)	41 (19)	0.56 (-5.12, +6.31)	0.84

10.4.3. Part C. Variability in urine volumes and urinary fluoride excretion between collections

Table 10.6 presents mean (SD) 24-h urine volumes (ml/d) and daily urinary fluoride excretion (mg/d and mg/kg bw/d) at two collections. The results showed no statistically significant differences between the two collections for either parameters.

Table 10.6 Urine volumes (ml/d) and urinary fluoride excretion (mg/d, mg/kg bw/d)

	Mean (SD) by method		**Mean difference (95% CI)**	**P value**
	2-day duplicate	3-day food diary		
urine volumes (ml/d)	516 (271)	482 (179)	+33 (-27, +94)	0.27
F excretion:				
- mg/d	0.368 (0.169)	0.373 (0.205)	-0.005 (-0.058,+0.048)	0.84
- mg/kg bw/d	0.018 (0.008)	0.018 (0.009)	0.00 (-0.002, +0.002)	0.96

The within-child variation in the rate of urinary fluoride excretion from test to re-test was ±0.146 mg/d (95% CI: 0.120-0.180 mg/d). After log transformation of variables from both collections and back transforming the data the within-child variability expressed as a ratio of the mean was ×/÷ 1.50.

10.4.4. Part D. Summary data

Since there was no significant difference in the main outcome variables (dietary fluoride intake, toothpaste ingestion and urinary fluoride excretion) between the methods/collections at the group level, the data were averaged (Table 10.7).

Table 10.7 Summary of main outcome variables for each method and the average of both methods

	Mean (SD)		
	3-day food diary	**2-day duplicate**	**Average of both**
Total F intake (mg/d)			
- Diet	0.533 (0.314)	0.583 (0.283)	0.558 (0.268)
• Contribution to TDFI (%)	54 (22)	57 (23)	55 (19)
- Toothpaste	0.568 (0.507)	0.648 (0.762)	0.608 (0.569)
• Contribution to TDFI (%)	46 (22)	43 (23)	45 (19)
Urine			
- Volume (ml/d)	482 (179)	516 (272)	499 (197)
- F excretion (mg/d)	0.373 (0.205)	0.368 (0.169)	0.371 (0.157)
- FUFE (%)	41 (26)	37 (22)	40 (18)
- DFR (mg/d)	0.618 (0.521)	0.740 (0.699)	0.679 (0.561)
- FFR (%)	49 (26)	53 (22)	51 (20)

10.5. Discussion

10.5.1. Fluoride intake

In this chapter fluoride intake from all dietary sources as well as the weight of these sources (food, drinks and water) was compared to investigate any difference that may exist between the two methods in the assessment of fluoride intake. In addition, expectorated saliva, toothpaste and rinse after brushing and urinary fluoride excretion were collected twice and compared to investigate variation in toothpaste ingestion and urinary fluoride excretion. No other study has attempted to compare these methods to the extent that this study has done.

A recent study in Indianapolis, USA, on small number of children (n=12) aged 15-30 month old has compared food diary and duplicate methods in estimating dietary fluoride intake (Martinez-Mier *et al.,* 2009). However, for the US children no data was reported on validation of dietary methods, toothpaste ingestion and urinary fluoride excretion.

- **Weight of food, drinks and water**

The results of this study showed a higher water and lower food consumption by children when dietary data were collected by 2-day duplicate method compared with when they were collected by 3-day food diary method. The observed differences in water and food consumption could partly be attributed to the week-to-week variation in food and drink consumption and partly to the differences in

methods of dietary assessment. While the type and pattern of food and drink consumption could be affected by season, in this study this effect was minimised by collecting dietary data within one week.

Food diaries can be completed easily due to their size (pocket size) and therefore food and drinks could be recorded at the time of consumption which consequently was less likely to be missed out. However, this method has some limitations in measuring accurate amounts of sips, bites and teaspoons which are recorded by participants. A post- collection interview was conducted using pictures and models to improve the accuracy of data collected by this method. Over-reporting has been suggested by some studies (Guha-Chowdhury *et al.,* 1996, Martinez-Mier *et al.,* 2009) which also was confirmed by the results of the present study (data for 3 children was over reported). On the other hand 2-day duplicate tends to under report due to the higher level of commitment from participants in terms of carrying the containers or removing the bones, stones, skins etc.

- **Dietary fluoride intake**

a) Group level

Despite the higher mean weight of food recorded in 3-day food diary method compared with the 2-day duplicate method, no meaningful difference was observed in the mean fluoride intake form this dietary source. However, there was a significant difference in dietary fluoride intake from all drinks (including drinking water) between the two methods which was mainly due to higher reported water consumption by duplicate method (Table 10.2).

The findings of the present study were in contrast to those reported for 15-30 month old US children by Martinez-Mier (Martinez-Mier *et al.,* 2009). For the US children higher fluoride intake was reported from food by food diary while the corresponding value from drinks was reported to be similar between the two methods. No data was reported on the fluoride intake from water as a separate source for the US children.

At the group level, the mean difference in the estimated total dietary fluoride intake by the two methods was -0.003 mg/kg bw/d (-0.05 mg/d). This difference is not meaningful considering that the lower threshold of optimal F intake is 0.05 mg/kg bw/d.

- **Individual level**

The Bland-Altman limits of agreement analysis and plot is usually used for evaluating a new treatment or method by comparing it with an established method. However, in this study both methods have already been established at the group level, but the agreement at individual level has never been evaluated. It is most unlikely that two different methods agree exactly, by giving the identical results for all individuals. One of the aims of this section was to find out the extent of agreement between these methods.

The mean difference of 0.641 mg/d in dietary fluoride intake between the two methods for one child was found to be above mean dietary fluoride intake estimated by each method (0.533 mg/d for 2-day duplicate and 0.583 mg/d for 3-day food diary method). Checking the validity of dietary data provided by this child showed that dietary data collected by the 3-day food diary method was over-reported which explains the higher mean difference. The difference could also be explained by the daily variation in dietary fluoride intake by individuals. Overall, the wide limit of agreement suggests that the methods cannot be used interchangeably at the individual level.

- **Daily variation in fluoride intake**

While seasonal variation in dietary fluoride intake has been reported by several studies (Zohouri & Rugg-Gunn 2000b, Levy *et al.*, 2001, Levy *et al.*, 2003, Broffitt *et al.*, 2004), there was only one report in the literature on daily variation in dietary fluoride intake in children (Martinez-Mier *et al.*, 2009).

Within-child between days variation in dietary fluoride intake was reported for the US children when dietary data collected by 3-day food diary (0.198 mg/d) and 3-day duplicate method (0.151 mg/d) (Martinez-Mier *et al.*, 2009). The corresponding figures in the present study at 0.280 and 0.190 mg/d for 3-day food diary and 2- day duplicate methods respectively were slightly higher than the US study. While children in the US study were recruited from a single day-care centre where they had identical food menus and limited choice of food, in the present study most children took their own packed lunch and snacks to schools with daily variation in types of food and drinks and the possibility of swapping their food/snacks with friends. In addition, in this study dietary data were collected on

both week days and week end days to cover any variation in dietary patterns between week days and weekends.

Between days, within-child variation in fluoride intake in this study was slightly higher by 3-day food diary method compared with that by 2-day duplicate method. These results are in agreement with those reported for 15-30 month old US children (Martinez-Mier *et al.,* 2009). One factor that could contribute to the lower variation obtained by 2-day duplicate method was difficulties in collecting duplicate samples.

Between individual variations in this study is consistent with previous studies (Clovis & Hargreaves 1988, Levy *et al.,* 2003), although it is difficult to compare the results of the present study to previous ones because of different methods used.

10.5.2. Variation in toothpaste ingestion

a) Group level

The present study found no statistically significant difference in the mean amount of toothpaste used and ingested per brushing at the group level between collections which was in agreement with previous studies reported for 3-5 year old Canadian children (Naccache *et al.,* 1990). However, for 4 year old Iranian children toothpaste ingestion was reported to be significantly different (39% and 45%) between the collections (Zohouri & Rugg-Gunn 2000b). For the Iranian children variation between the collections was attributed to the use of different types of toothpaste in each collection. Whereas in this study 87% of children used the same brand of toothpaste in both episodes.

b) Individual level

The typical within-child variation in toothpaste ingestion in this study was substantial (×/÷ 1.50). Within-child variation in toothpaste ingestion has also been reported for 3-5 year old Canadian children (Naccache *et al.,* 1990). For both Canadian and British (present study) children this variation was random. In one occasion the child may expectorate, swallow and use different amounts of toothpastes to the other occasion.

The current study also found a large between-child variation in toothpaste ingestion. This could be explained by the wide range in fluoride concentration of toothpastes used among the current study population (250 to 1450 ppm), the

amount of toothpaste used, and other tooth brushing habits such as expectorating and rinsing.

10.5.3. Urinary fluoride excretion

- **Urine volumes**

In the present study no significant difference was observed in the urine volumes between the two collections. Urine volumes were reported to be different between summer and winter in 3-5 year old Australian children (Crosby & Shepherd 1957), which was due to the difference in the amount of drinks consumed in each season. Whereas for 4 year old Iranian children no significant difference was reported in the urine volumes between the seasons despite the fact that higher amounts of drinks were consumed in the summer (Zohouri & Rugg-Gunn 2000b). However, in this study seasonal variation did not apply due to collections within one week.

- **Daily variation in fluoride excretion**

a) Group level

Current study found no significant difference in the mean 24-h urinary fluoride excretion between the two collections at the group level. Indeed, when it was expressed on the body weight basis, both collections showed similar results (0.018 mg/kgbw/d).

The findings of the current study are in agreement with the Iranian study on 4-year children which reported no difference in the mean urinary fluoride excretion between the two seasons (Zohouri & Rugg-Gunn 2000b). No other data was available in the literature to report variation in the urinary fluoride excretion.

- **Individual level**

A large within-child variation was observed in the rate of urinary fluoride excretion in this study. No data was available in the literature to report within-child variation in the urinary fluoride excretion. This is the first study to report this variation in a relatively large number of children living in optimally fluoridated areas. Since the measurement errors in the analytical methods used for urine samples was not significant (0.022 mg from test to re-test) the large variation in the present study could be explained by: a) variation in beverage consumption particularly those diluted with water, b) variation in toothpaste ingestion as highlighted in the previous section and finally c) variation in the

composition of diet which could affect urine pH and consequently fluoride excretion (Whitford 1990).

10.5.4. Average of both methods

The average data showed that the contribution of diet and toothpaste to total fluoride intake was almost equal. The average fraction of fluoride excreted in urine was within the range of 30-40% reported for children (Murray *et al.,* 1991).

10.6. Conclusion

From the results of this chapter it can be concluded that:

1. Mean difference in estimated daily dietary fluoride intake between the two methods was not significant at the group level. Therefore, either method can be used when estimating mean daily dietary fluoride intake of a population. However, since difference in dietary fluoride intake from the individual dietary sources was significant the methods cannot be replaced when investigating fluoride intake from individual dietary sources.
2. At individual level, the agreement between the two dietary assessment methods in estimating dietary fluoride intake was poor, indicating that the methods cannot be used interchangeably at individual level.
3. The results showed a significant variation in dietary fluoride intake between days which should be taken into account when measuring daily fluoride intake by increasing the number of days to collect dietary data.
4. The within and between children variation in toothpaste ingestion was also large. These findings suggested that one tooth brushing collection could not represent the constant and usual fluoride intake from this source.
5. A large within-child and between children variation in urinary fluoride excretion in the present study also suggested that studies using urinary fluoride excretion as a biomarker of fluoride exposure should not rely on one collection only.

Chapter 11 Overall Discussion and conclusion

11.1 Introduction

This chapter presents an overall discussion from all chapters. The discussion concerns the study design, recruitment, employed dietary methods and their abilities to provide valid dietary data on fluoride intake. The study is then concluded and closed with contribution to the current knowledge and further suggestions for future research.

11.2. Design, locations, subjects and recruitment

This study was conducted in a fluoridated area to evaluate the ability of dietary assessment methods in estimating fluoride intake.

Since the calcification of the crown of most permanent teeth occurs during the first 5 years of life (Fomon *et al.,* 2000) and due to practicality of data collection the age group of 4-6 year old was selected in the present study.

The sample size was estimated based on the confidence interval derived from the difference in mean total dietary fluoride intake resulted from each method. The desired precision of estimation was the mean difference ±1/3 of the standard deviation with a two-sided 95% confidence interval. This resulted in a required sample size of 44 participants. Allowing a 30% attrition rate resulted in a total sample size of 60.

Giving the age range selected for this study, targeting primary schools was considered the best approach for the recruitment of individuals. Recruitment of young children through schools is a difficult task and previous studies of this nature conducted in developed countries reported problems regarding the co-operation and support from the head teachers. A UK based study aimed to measure urinary fluoride excretion of 3-5 year old children drinking fluoridated milk (Ketley & Lennon 2001), reported a non-random selection of the schools with the supports and co-operation from the head teachers. In the present study, the participated schools were also those agreed to take part. The overall response rate was 28% of the total number of schools invited. All families were contacted through the schools and the response rate was 7%. Limitation of time and resources could be the major reasons for low recruitment rates in most of the research of this nature. All of the 61 children who took part completed all aspects

of the study, despite the difficult nature of the study which required the co-operation of parents for their close supervision of their child's diet in order to complete food diaries or collect duplicates of food, as well as collection of two 24-h urine samples. Sufficiently informing parents, face-to-face discussion, flexibility and regular contact with parents during the study were the key elements to maintain their involvements. In addition, provision of incentive as one of the motivating factors should not be underestimated.

11.3. Fluoride intake

11.3.1. Dietary fluoride intake and validation of dietary methods used for measuring fluoride intake

All dietary assessment methods have inherent errors and shortcomings which make their validity assessment a challenge. Obtaining accurate information concerning children's diet from multiple sources is difficult and with repeating measurements the possibility of misreporting increases (Westerterp & Goris 2002). No fluoride intake study has reported the validity of the employed dietary methods. The results of the validation in this study showed a likelihood of under-reporting of food intake by the duplicate method (15 out of 61 children). Under-reporting by duplicate method has also been reported in other studies measuring other nutrients intake (Somogyi & Kopp 1983, Knuiman *et al.*, 1987). In addition, duplicate plate technique could influence usual food intake as some people may think it is wasteful to put food in a bucket merely for the purpose of chemical analysis and may decide to eat less expensive food (Knuiman *et al.*, 1987). In contrast, dietary data recorded by the 3-day food diary method in this study showed three cases of over-reporting.

In the present study both methods provided almost similar results on total dietary fluoride intake at the group level (0.533 and 0.583 mg/d). However, at the individual level, a wide range of dietary fluoride intake from 0.099 to 1.391 mg/d for 2-day duplicate method and 0.135 to 1.808 mg/d for 3-day food diary method was observed.

Both methods provided information on fluoride intake from major dietary sources (i.e food, drinks and water). However, food diary method provided more detailed information on sources of fluoride intake from subgroups such as boiled

vegetables, cooked rice, pasta, etc, as well as their contribution to daily dietary fluoride intake (Table 8.3).

According to the parents' feed backs food diary was the preferred method by the majority. It appeared that this method did not interrupt their normal dietary habits. They also found that the method was less time consuming, easy to use and economic since no food was wasted as a result of duplicating.

In most studies including the present study the cost of duplicated foods has been compensated to the participants. Therefore, it might not be suitable for the large epidemiological studies.

11.3.2. Toothpaste ingestion

In this study a questionnaire was used to collect information on tooth brushing habits of the children. Samples of expectorated saliva tooth brushing and rinse were collected from children to assess fluoride intake from toothpaste ingestion. The results showed that fluoride intake from toothpaste was significantly influenced by the amount of toothpaste used, fluoride concentration of the toothpaste and the age of the child. The results of this study were in line with the previous studies which showed a direct correlation between the amount of toothpaste loaded and fluoride ingested and the inverse correlation between age and the toothpaste ingestion (Tan & Razak 2005, de Almeida *et al.*, 2007, Kobayashi *et al.*, 2011). The greater tendency to swallow the toothpaste by younger children is due to poor control of the swallowing reflexion. Therefore, it is important to reinforce supervision of young children by parents when brushing their teeth, use a pea-size amount and toothpaste with lower fluoride concentration. Habits of expectorating, rinsing and toothpaste flavour are among the other variables which could contribute to the degree of toothpaste ingestion by children (Kobayashi *et al.*, 2011).

The current study showed substantial within-individual variation in toothpaste ingestion which could be a result of variation in expectorating, rinsing, and the amount of toothpaste used as well as the fluoride concentration of the toothpaste.

11.4. Total fluoride intake

Total daily fluoride intake estimated by the two dietary assessment methods was very close: 0.05 mg/kg bw/d by the food diary and 0.06 mg/kg bw/d by the

duplicate method. The contribution of toothpaste to total daily fluoride intake was also similar between the collections; 52% (along with 3- day food diary method) and 53% (along with 2-day duplicate method).

It has to be noted that tooth brushing samples were not collected on the diet and urine collection days and at the normal brushing times (i.e. mornings and evenings) due to the practicality of sample collection at those particular times. Therefore, the calculated amount of toothpaste ingestion in this study was not the actual fluoride ingestion from toothpaste on the same day when the dietary data and urine samples were collected. This was considered as one the limitations of this study. Timing of tooth brushing collection has not been reported in previous studies.

11.5. Urinary fluoride excretion

According to the validity criteria employed in the present study, no urine sample was excluded due to incomplete collection. On average, the volume of 24-h urine samples in this study was 499 ml/d. The mean estimated urinary fluoride excretion (0.371 mg/d) in this study was within the WHO provisional standards of 0.360 to 0.600 mg/d for 3-7 year old children living in optimally fluoridated areas. Due to difficulties in obtaining accurate dietary data, some studies have attempted to estimate daily fluoride intake from urinary fluoride excretion by establishing an association between intake and excretion. In children who are in active growth and have high bone turnover, it has been suggested that between 30-40% of the ingested fluoride is excreted in the urine (Murray *et al.,* 1991). However, studies in children reported a wide range of urinary fluoride excretion from 17% (Ekstrand *et al.,* 1994) to 84% (Brunetti & Newbrun 1983). The overall fractional urinary fluoride excretion of 40% obtained in this study was found to be within the above range.

11.6. Conclusion

The main aims of the study were to develop a better understanding of strengths and weaknesses of the 2-day duplicate and 3-day food diary methods and evaluate the validity of each method for estimating dietary fluoride intake in young children.

In line with the main aim of the study it was concluded that:

- The 2-day duplicate method had a greater tendency towards under-reporting food intake. The cost and availability of enough food at home for duplicating contributed to under-reporting in the duplicate method. In addition the dietary habits were more likely to be altered when this method was employed. In contrast, when dietary data were collected by the food diary method, the possibility of over-reporting was greater whereas no under-reporting was observed. One source of error in both methods was found to be the estimation (not weighing) of food portions.
- There are many potential sources of errors in any dietary assessment method, which could cancel each other at the group level. However they might well introduce a large source of error at the individual level.
- At group level, mean total daily dietary fluoride intake estimated by the 3-day food diary method was very close to the corresponding value measured by the 2-day duplicate method. Therefore, either method can be used when estimating mean total daily dietary fluoride intake of a population.
- The agreement between the two methods in estimating total daily dietary fluoride intake at the individual level was poor, indicating that the methods cannot be used interchangeably at the individual level.
- The significant day-to-day variations in dietary fluoride intake suggest that dietary data should be collected for more than one day and include week-day and weekend-day.
- The large within-child between collections variation in toothpaste ingestion imply when investigating total daily fluoride intake, more than one sample should be collected. Also, when investigating fractional urinary fluoride excretion, the expectorated saliva, toothpaste and rinse sample should be collected on the same day as diet and urine collection.
- The large within-child between collections variation in urinary fluoride excretion also suggest that studies using urinary fluoride excretion as a biomarker of fluoride exposure should not rely on only one collection.
- The mean total daily fluoride intake (diet and toothpaste) suggest that the children, as a group, received the so-called recommended intake of

fluoride (0.05-0.07 mg/kg bw /d). However, total daily fluoride intake of more than half of the children, when investigated individually, was below the recommended daily intake of fluoride for effective protection against caries.

- In a fluoridated area under customary fluoride intake conditions, on average 40% of the ingested fluoride by 4-6 year old children was excreted in the urine.

11.7. Suggestions for further research

11.7.1. Cost effectiveness of dietary assessment methods

Degree of burden that each dietary assessment method puts on participants could influence the co-operation and motivation for recording/collecting dietary information. Time and cost were recognised as the two major burdens by participants as well as by the researcher in this study. Both time and cost are important issues which should be taken into consideration when designing large epidemiological studies. An economic evaluation comparing these two dietary methods is therefore required to investigate the costs and benefits of each method. A qualitative study to investigate the acceptability of each method by parents and their children as well as their choice could also benefit future studies.

11.7.2. Development of a comprehensive fluoride database

While "food composition tables" such as McCance and Widowson (the UK food composition table) provide nutritional values of food and drinks including details of energy, protein, fat, vitamins and minerals, they do not contain any data on the fluoride contents of food and drinks to facilitate estimation of dietary fluoride intake. A comprehensive fluoride database which is nationally representative of fluoride concentration of food and drinks could assist future fluoride exposure studies. Therefore, there is a need to develop a UK-based fluoride database which can be used for larger epidemiological studies.

11.7.3. Establishing a reference range for nitrogen and potassium ratios in children

Biomarkers of urine provided useful assessment tool for validating dietary assessment methods in this study. The ratios of UN/DN and UK/DK were

calculated and used to validate dietary data for children. A range between 20-100% was proposed as acceptable range for both ratios in this study. The importance of these biomarkers in relation to validation of dietary methods in such a group of children warrants further examinations and provision of a robust reference range for different age groups.

11.7.4. Fluoride excretion range

Monitoring fluoride exposure should be carried out prior to and during any fluoride supplementation programmes in order to measure levels of fluoride exposure. Guidance for monitoring fluoride exposure at community level was developed by the WHO in 1999. However, their provisional guidance/standards are based on total daily urinary fluoride excretion of two broad age groups without considering the body weight of children. Since 1999, more data on fluoride excretion of children have been published. Therefore, it is important to review the suggested WHO guidelines in view of the recent publications.

Since recommendations on optimum fluoride intakes are based on body weight, establishing a urinary fluoride excretion reference range based on body weight is essential for studies of monitoring fluoride exposure.

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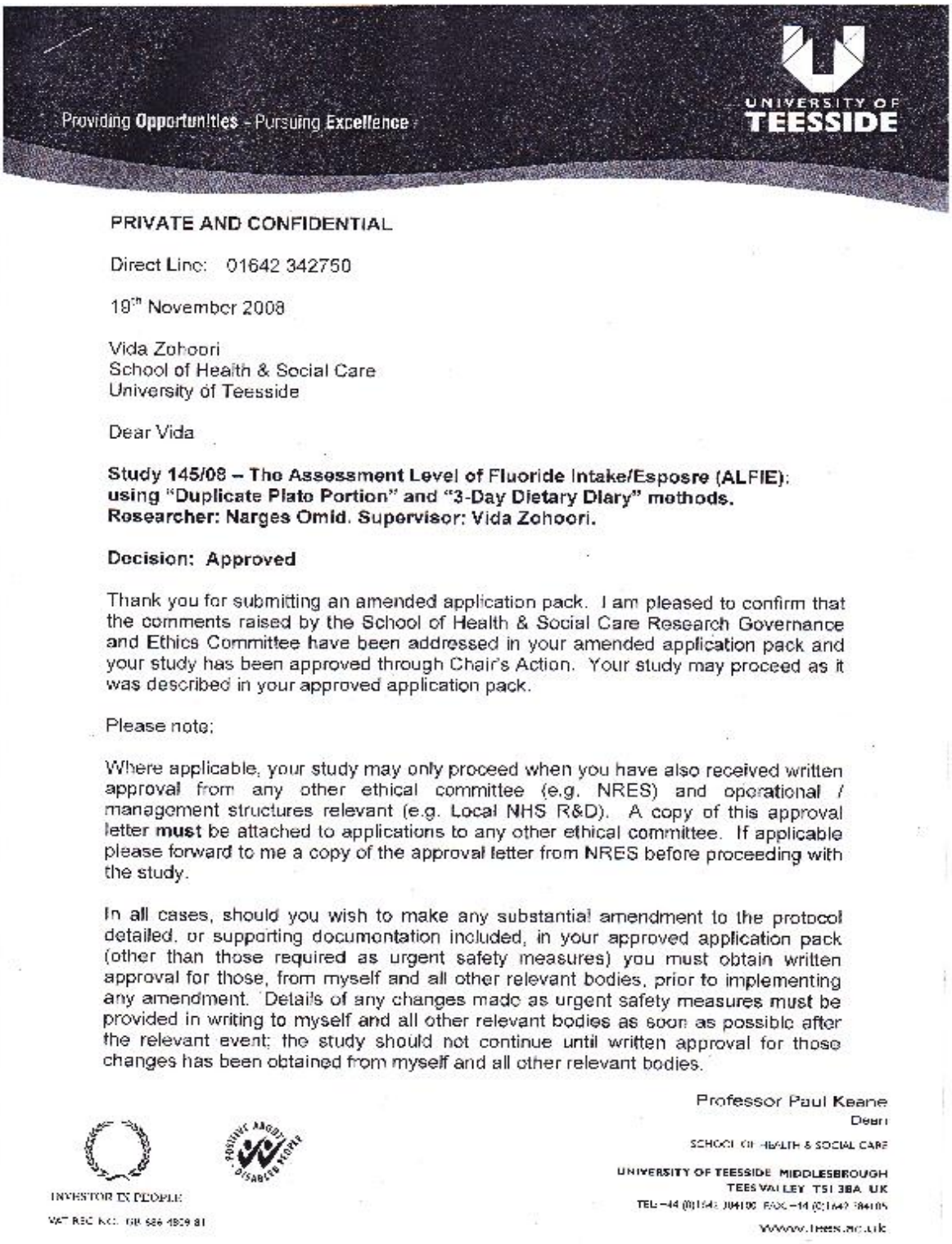
Appendices

Appendix 1 Ethics approval from school of Health & Social Care, Teesside University



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19th November 2008

Vida Zohoori
School of Health & Social Care
University of Teesside

Dear Vida

Study 145/08 – The Assessment Level of Fluoride Intake/Esposre (ALFIE): using "Duplicate Plate Portion" and "3-Day Dietary Diary" methods. Researcher: Narges Omid. Supervisor: Vida Zohoori.

Decision: Approved

Thank you for submitting an amended application pack. I am pleased to confirm that the comments raised by the School of Health & Social Care Research Governance and Ethics Committee have been addressed in your amended application pack and your study has been approved through Chair's Action. Your study may proceed as it was described in your approved application pack.

Please note:

Where applicable, your study may only proceed when you have also received written approval from any other ethical committee (e.g. NRES) and operational / management structures relevant (e.g. Local NHS R&D). A copy of this approval letter **must** be attached to applications to any other ethical committee. If applicable please forward to me a copy of the approval letter from NRES before proceeding with the study.

In all cases, should you wish to make any substantial amendment to the protocol detailed, or supporting documentation included, in your approved application pack (other than those required as urgent safety measures) you must obtain written approval for those, from myself and all other relevant bodies, prior to implementing any amendment. Details of any changes made as urgent safety measures must be provided in writing to myself and all other relevant bodies as soon as possible after the relevant event; the study should not continue until written approval for those changes has been obtained from myself and all other relevant bodies.

Professor Paul Keane
Dean

SCHOOL OF HEALTH & SOCIAL CARE

UNIVERSITY OF TEESSIDE MIDDLESBROUGH
TEES VALLEY TS1 3BA UK
TEL +44 (0)1642 384100 FAX +44 (0)1642 384105

www.tees.ac.uk

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On behalf of the School of Health & Social Care Research Governance and Ethics Committee please accept my best wishes for success in completing your study.

Yours sincerely

Dr. Alasdair MacSween
Chair
Research Governance and Ethics Committee
School of Health & Social Care

Appendix 2 Ethics Approval from County Durham & Tees Valley 1 Research Ethics Committee

NHS

National Research Ethics Service

County Durham & Tees Valley 2 Research Ethics Committee
Professorial Unit of Surgery
The Tatchell Centre
University Hospital of North Tees
Hardwick Road
Stockton-on-Tees
TS19 8PE

Telephone/Fax: 01642 624164

22 January 2009

Mrs Narges Omid
Full time PhD student
University of Teesside
School of Health and Social Care
Parkside West Offices
Middlesbrough
TS1 3BA

Dear Mrs Omid

Full title of study: **The asssessment Level of fluoride intake/exposure using "duplicate plate portion" and "3-day dietary diary" methods**

REC reference number: **08/H0905/116**

Thank you for your letter of , responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA). The favourable opinion for the study applies to all sites involved in the research. There is no requirement for other Local Research Ethics Committees to be informed or SSA to be carried out at each site.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission at NHS sites ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Anthropometric & Demographic data questionnaire		27 November 2008
Instructions on how to duplicate food & Drinks		27 November 2008
Instructions on how to collect urine samples		27 November 2008
Copy of BioCOSHH form		27 November 2008
Copy email fromHTA		27 November 2008
Letter of invitation to participant	V:1	27 November 2008
Questionnaire: Validated Food Diary		
Protocol	V:1	27 November 2008
Investigator CV		27 November 2008
Application	V:2	27 November 2008
CV Supervisor		27 December 2008
Letter of invitation to participant	V.1	27 November 2008
Response to Request for Further Information		
Participant Consent Form	V:4	01 January 2009
Participant Information Sheet	V:2	01 January 2009
Compensation Arrangements		01 August 2008
Covering Letter		12 January 2009

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review –guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our

service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

08/H0905/116 **Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Carol

Dr J Drury
Chair

Email: carol.cheesebrough@nhs.net

Enclosures: "After ethical review – guidance for researchers" SL-AR2

Copy to: Professor Paul Keane R&D Dept., University of Teesside

Appendix 3 Information on the division of city into Wards and Bands

Ward Analysis			
City Rank	**Ward**	**IMD 2004 Score**	**City Group (5 Bands)**
21	Westerhope	16.38	4
22	West Gosforth	13.69	5
23	Dene	11.51	5
24	East Gosforth	11.01	5
25	North Jesmond	10.21	5
26	Parklands	10.17	5
1	Walker	66.37	1
2	Elswick	63.67	1
3	Byker	58.45	1
4	Benwell and Scotswood	50.63	1
5	Westgate	48.39	1
6	Kenton	41.14	2
7	Blakelaw	40.04	2
8	Woolsington	39.98	2
9	Fawdon	38.97	2
10	Wingrove	36.82	2
11	Denton	34.28	3
12	Lemington	33.85	3
13	Walkergate	33.72	3
14	Fenham	33.01	3
15	Newburn	31.87	3
16	Ouseburn	29.97	3
17	South Heaton	28.88	4
18	Castle	18.26	4
19	South Jesmond	17.00	4
20	North Heaton	16.90	4

Appendix 4 Letters to Head teachers

Providing **Opportunities** - Pursuing **Excellence**

UNIVERSITY OF TEESSIDE

Mr Barrie Russell
Welbeck primary School
Flodden Street
Walker
NE6 2QL

Date: 01/05/09

Dear Mr Russell,

The Assessment Level of Fluoride Intake/ Exposure (ALFIE) using "2-day Duplicate Plate Portion" and "3-day Dietary Diary" methods.
Researcher: Mrs Narges Omid (Research Student)

We write to request your help in facilitating some research which we hope to carry out in some Newcastle schools. We would like to gather information to enable development of a standard assessment tool for measuring fluoride intake.

This study is being carried out by Mrs Narges Omid, a PhD student at the University of Teesside, and has ethical approval from the Local Research Ethics Committee (a copy of the approval is enclosed). The researcher also (Narges) holds an enhanced CRB disclosure.

As you may know, fluoride has important dental health benefits, through systemic use in fluoridated water as well as in foods and drinks, which contain fluoride naturally, and also through topical use with toothpastes and other oral health products. While fluoride at low concentrations helps protect teeth against dental decay, some 'discoloration' or 'mottling' of teeth can sometimes occur if young children consume excessive amounts of fluoride through toothpaste ingestion or their diet. Therefore, regularly monitoring fluoride intake has been recommended by many organisations including the World Health Organisation. Despite the importance of monitoring issues, there is no standard universally accepted method for evaluating fluoride intake. The study we plan to carry out will compare the two most commonly used methods of measuring fluoride intake in children ("duplicate plate portion" and "3-day dietary diary") in order to develop a better understanding of the strengths and weaknesses of the two methods. By evaluating the reliability and validity of these methods, their suitability for assessing fluoride intake in 4-6 year old children can be determined.

The study will also provide information on fluoride intake and excretion of 4 to 6 year old children who have lived continuously in fluoridated areas of the North-East of England.

Professor Paul Keane
Dean
SCHOOL OF HEALTH & SOCIAL CARE
UNIVERSITY OF TEESSIDE MIDDLESBROUGH
TEESVALLEY TS1 3BA UK
TEL: 44 (0)1642 384100 FAX: +44 (0)1642 384105
www.tees.ac.uk

Your school has been selected randomly from a list obtained from Education Directorate and we write to ask if you will consider becoming involved. With your permission we would like to ultimately recruit all of 4-6 year old children from your school.

The study is likely to fit in with your School's curriculum and with this in mind, we would be happy to visit you again after the study and tell you what we found out. For each returned response form a £1 book voucher will be given to schools.

Each child will need to have a food diary kept for them by their parents for a total of 3 days, including 2 week-days (Thursday and Friday) and one weekend day (Saturday) for one method. In addition, for the second method, the diet of each child will be duplicated by parents for a total of 2 days including 1 week-day (Friday) and one weekend day (Saturday). However for the second method, if a child is having a school dinner on a Friday, we would like to arrange with your school, to purchase the same meal as we need a duplicate for analysis. Should you agree, Narges would be at your school over one lunch time to observe and collect the food portion for any child who is having a school dinner. We will check with parents of children who take packed lunches to school for any swapped or purchased items at school. The parents will also be asked to collect their children's urine for two 24 hour period on two Saturdays to enable us to measure their fluoride excretion and we will be collecting samples of saliva spat out during toothbrushing. Narges would arrange with parents to collect all the samples from the children's homes, and store them in a freezer for analysis.

For your information, we enclose with this letter, a pack containing a copy of the parent information booklet, food diary, duplicate collection instruction and urine collection information/instruction sheet which we would like to distribute to the parents of all children aged 4 to 6 years prior to the study to allow them to decide if they would be happy to consent to their child taking part. With prior arrangement with you Narges will be in your school to distribute the packs at the end of a lesson and then be on hand the next day to answer any questions the parents may have.

We will contact you over the next few days to see if you are interested in your school taking part. If so, Narges would then come and talk to you more about the study and answer any questions you may have.

In the mean time if you have any questions which you would like to ask please do not hesitate to contact us on the number listed below.

Yours Faithfully,

Dr V Zohoori
Senior Lecturer in Research
School of Health & Social Care, University of Teesside
Tel: 01642342973
Research Mobile No: 07925153789

Appendix 5 Letters to parents

Professor Paul Keane obe
Dean
School of Health & Social Care
Teesside University, Middlesbrough
Tees Valley TS1 3BA UK
T: +44 (0)1642 384 620
F: +44 (0)1642 384 621
www.tees.ac.uk

Teesside University

Date

Dear Parent/Guardian,

We are writing to invite you to take part in a research being carried out by the School of Health and Social Care, Teesside University.

In this study we are looking at the amount of fluoride which is taken by your child and the amount excreted in urine.

This study involves collection of your child's diet by two dietary collection methods "3-day food diary" and 2-day duplicate plate". You are asked to complete a food diary for your child for three consecutive days for one occasion and collect the same amount of food and drinks your child consumes for two consecutive days in another occasion. In addition you will be required to collect two 24 hour urine samples from your child starts on a Saturday. Further details about this study are provided in the enclosed study information document. We have also included a response form to return to your child's school.

We will happily give you a £55 gift voucher to minimise the inconvenience of cost involved in duplicating your child's diet as well as to thank you and your child for helping to complete the study.

The research student Mrs Narges Omid will be in your child's school on to answer any question and concern you might have with regard to any aspect of the study.

We hope you will consider taking part and looking forward to hearing from you.

Yours Faithfully

Dr Vida Zohouri

Appendix 6 Study Information Document

The Assessment Level of Fluoride Intake/Exposure (ALFIE) using "2-day Duplicate Plate Collection" & "3-day Dietary Diary" methods

Study Information Document (Parents/Guardians)

School of Health and Social Care
University of Teesside
Middlesbrough

What is this Study Information Document (SID) for?

This SID provides information regarding this study and invites you to take part in this research project.

Who is doing this research?

This is part of a PhD project. The researcher is Mrs Narges Omid, a PhD student from Teesside University who is supervised by Drs Vida Zohoori and Alan Batterham from School of Health and Social Care and Dr Liam O'Hare from the School of Science and Technology, University of Teesside and Dr Anne Maguire from the School of Dental Sciences, Newcastle University

What are we researching?

We are trying to compare the 2 most widely used methods of measuring fluoride intake in children in order to develop a better understanding of strengths and weaknesses of the two methods and therefore evaluate their validity and suitability for assessment of fluoride intake in 4-6 year old children.

By finding this out, we will be able to provide valuable information for future work in fluoride research, particularly fluoride exposure. Also, as secondary aims of thresearch, we would like to know how much fluoride is taken in by children (from the food and drinks they consume) and how much is excreted into their urine.

Why are we researching this and what are the benefits?

Fluoride has an important role in developing and maintaining healthy teeth especially during the first 5 years of life. It is found naturally in foods we consume, in water we drink as well as in most toothpaste we use. While fluoride at low concentrations helps protect teeth against dental decay, 'discoloration' or 'mottling' of teeth can occur if young children are exposed to excessive levels of fluoride. Therefore, regular monitoring of fluoride intake has been recommended by many organisations including the World Health Organisation. Despite the importance of monitoring the intake of fluoride, there is no standard universally accepted method for measuring fluoride intake. This study will help in the development of a standard assessment tool for measuring fluoride intake in children. As a result we will be able to monitor deficient or excessive intake of fluoride more accurately.

Why have we asked your child?

Your child has been asked because their school is in Newcastle which has

received a fluoridated water supply for over 40 years.

Can your child participate?

Your child can take part if he/she is
-Between 4-6 years old;
-Has no dietary restrictions;
-Has good general and oral health;
-Has been living in a fluoridated area (Newcastle) since birth;
-Able and available to take part in both procedures.

However, your child can not take part if he/she is:
-Using medicines;
-Has restricted diet
-Has gastrointestinal, bone, renal problems or history of urinary tract infections
-Has any other illnesses such as chest infections, temperature, etc.
Information about your child's health will be asked once you agree to take part

What will happen if you and your child decide to take part?

If you and your child decide to take part, you will be asked to:
1) Keep a record of everything your child eats and drinks for 3 consecutive days.
2) Duplicate food/drink that your child consumes for 2 consecutive days. .
3) Collect your child's urine for two full 24 hour periods.
In addition, we would like to collect the saliva and toothpaste your child spits out during brushing of their teeth.

Will you need any special equipment?

We will provide all the equipment that you will need, and clear instructions on how to collect the information we need. Narges would be present to do the tooth brushing sample collection with your child.

Urine collection sounds hard!

It is not though; we will provide you with a jug to collect your child's urine in and also a large screw top bottle in which to store it. The urine can be kept in the

bathroom by the toilet and does not need any special storage. The bottle will be collected from you by Narges.

What about the 3 day food diary?

We will give you a special diary in which to record the food and drink your child consumes with full instructions on how to fill in the diary. You will not be required to weigh the foods, just to give us an idea of the portion by writing it down in the diary, e.g. an Asda cod fish finger, small glass of water. The food diary needs to cover two weekdays and one weekend day.

What about the duplicate food and drink samples?

We need exact duplicates of all the food and drinks which your child eats or drinks over 2 days. We will give you instructions on how to provide the duplicate food and drink samples as well as a demonstration on how you should collect them. We will also provide you with containers for the food and drink items and will collect them from you. If your child takes a school dinner on a Friday, Narges will be at your child's school during lunch time to observe your child eating dinner and to remove and collect any leftover once they are finished.

How is the saliva collected?

When Narges comes to pick up the urine collection she will also ask your child to brush their teeth. She will weigh the toothpaste used and collect the saliva and toothpaste which is spat out in a container for analysis later.

Do we need other information?

Yes, we will also be measuring your child's height and weight, asking about their tooth brushing habits and recording their post code, date of birth and school.

Will the information be kept confidential?

Yes, you and your child's details including home address will be kept confidential. Your child will be given an ID number on the consent form and the information sheet. This number will be used to keep all information collected anonymous. Your child's name will not be recorded with the data collected. All the information collected will be completely anonymous and held securely in the strictest confidence at the University of Teesside.

What is your involvement?

You and your child will be visited four times at home to provide you with the essential information and collection materials and then obtain the

information described above and collect the samples.

Visit one:
We will visit you before starting the study to ask you some general questions including your child's date of birth and age, your child's general health, your child's tooth brushing habits such as type of regularly used toothpaste, starting age of using toothpaste, frequency of brushing per day.
In this visit, we will also 1) measure the height and weight of your child, 2) give you either a food diary or appropriate containers and tubes to collect food and drink samples; and explain in detail how to complete the dietary diary or demonstrate how to collect the duplicate plate foods; and 3) give you plastic urine collection bottles, a funnel, jug and disposable container to collect your child's urine samples and explain in detail how to collect the urine samples.
This visit might take up to one hour.

Visit two:
We will visit you again after you complete the food diary or collect the food samples. In this visit, we will go through the food diary with you and your child to make sure that we have all the necessary information. We will also ask your child to brush their teeth according to their usual habits and collect toothpaste and saliva spat out after brushing. Your child's urine sample as well as food and drinks samples will also be picked up at this visit so it might also take up to one hour.
We will visit you again after 3 to 4 months of the first visit. If you were given a food diary to complete on the first visit, this time you will be asked to provide a duplicate plate of food and vice versa.

Do you have to take part?

No; your participation would be entirely voluntary. The researcher, Mrs Narges Omid, from the University of Teesside will describe the study and go through the information sheet with you. If you agree for you and your child to take part you will be asked to sign a consent form. You can withdraw from the study at any stage up to and including the date of the final visit without giving a reason. To withdraw from the study, you can call Narges Omid - the researcher – her contact details are shown below) and quote your child's ID number which can be found on your consent form and the information sheet.

Do you need more information before taking part in the study?

Narges Omid will be in your child's school on

…….at…….. when she will be happy to answer any questions you may have.

Finally….

We do understand that this study involves quite a lot of input from you as well as the inconvenience of the collection of your child's urine and an additional cost to duplicate your child's diet, therefore we would like to thank you at the end of the study by providing you with a £55 gift voucher (£40 for 2-day duplicate food collection, £10 for two 24-hour urine samples provided and £5 for recording a 3-day dietary diary).

If you have any questions after taking part in the study:

If you are concerned about any aspect of the study, you can call Narges (Research Mobile No: 07925153789) who will be happy to answer any questions you might have. Should you still have any concerns, you can contact Dr Vida Zohoori on 01642342937 at the School of Health and Social Care, University of Teesside.

Thank you for taking time to read this information

Appendix 7 Response form

Response form

Please tick as appropriate:

My child and I would like to take part in the study ☐

My child and I would like to receive more information before agreeing to take part in the study. ☐

my child and I are NOT interested in taking part in this study. ☐

Parent/ guardian's name:……

Child's name:……

Parent/child's address…….

……

……

Tel:.....

Mobile:……..

Parent/guardian's Signature:…….

Appendix 8 Consent form

CONSENT FORM (Parent/Guardian for child))

Name of Research Project:
The Assessment Level of Fluoride Intake/Exposure (ALFIE): using "Duplicate Plate Portion" and "3-Day Dietary Diary"
Name of Chef Investigator: Mrs Narges Omid
Name of Child:……Male/Female

Date Of Birth: …….

- All information collected and used for the purpose of this study will remain anonymous and confidential. The information will be collected and stored on paper and on computer. The paper documents will be stored in a locked filing cabinet and the researcher will be the only person with access. The computer documents will be stored on a password protected computer in password protected files. All information will be stored at University of Teesside.

- Please read the following questions carefully. If you answer "yes" to the question, please put your initial the box (on the same line as the question). Please repeat this for each of the questions asked.

		Please initial box
1.	I have read and understood the information sheet for the above study and had time to think about it.	☐
2.	I understand that my child's participation in this study is entirely voluntary.	☐
3.	I understand that I can withdraw my consent at any stage without giving reason and without prejudice.	☐
4.	I understand that all information will be treated as confidential, and that my child will not be identified in any way.	☐
5.	I understand that by signing and returning this form, I am giving my consent for my child to participate in this study.	☐
6.	I have explained to my child the processes involved in this study	☐

If you have answered "yes" to all of the above questions, please complete the details below:

Parents/guardian name: ……..

Parents/guardian signature…… Date……

I can confirm that I have explained to the participant the nature of this study and have given adequate time to answer any questions concerning it.

Signed…… Date…….

If you have any questions then you can ask Mrs Narges Omid on Research Mobile No:

07925153789

School of Health & Social Care, University of Teesside.

THANK YOU for agreeing to take part in this research
When completed : 1 copy to participant, 1 copy for researcher file

Appendix 9 Data collection form (Demographic and tooth brushing)

Child Code | | | |

Demographic data:

First name: ……...

Surname: …. …….

Gender: ……..

Date of Birth: ……

Home address: ……

…….

…….

School: ……..

Anthropometric data:

Weight (kg): ……

Height (cm): ……..

Tooth brushing habits:

Starting age of using a dentifrice (Yrs and Months)……

Type of regularly used toothpaste: (Brand and name)……

Frequency of brushing per day: (No. times per day) ……..

The person who brushes child's teeth……

The person who puts toothpaste on the toothbrush…….

Appendix 10 Randomisation list

Subject ID	Subject Name	Method	Subject ID	Subject Name	Method
1		A	31		B
2		A	32		A
3		B	33		B
4		B	34		B
5		A	35		A
6		B	36		A
7		A	37		A
8		B	38		A
9		A	39		B
10		B	40		B
11		B	41		A
12		A	42		B
13		B	43		B
14		B	44		A
15		A	45		A
16		A	46		B
17		B	47		B
18		A	48		A
19		B	49		B
20		A	50		A
21		A	51		B
22		A	52		A
23		B	53		A
24		B	54		A
25		B	55		B
26		A	56		B
27		A	57		A
28		B	58		B
29		A	59		B
30		B	60		A
			61		A

Appendix 11 The first two pages of food diary which include instruction and an example of a one day record

The Assessment Level of Fluoride Intake/Exposure (ALFIE) using "2-day Duplicate Plate Collection" &
"3-day Dietary Diary" methods

Food diary

Child ID number: ……

Record Days: Thursday……

Friday……

Saturday……

Instructions

☺ Please remember to carry this diary with you everywhere you go for 3 days.

☺ Write down EVERYTHING your child eats and drinks each day.

☺ Use a new line for each food or drink.

☺ Give as much detail as possible stating the name of the food/drink, brand, flavour, cooking method or preparation (for drinks such as squash, milk tea, hot chocolate), and the time consumed.

☺ Give the amount of the food eaten:

- Drinks as glasses, cups or mugs.
- Breakfast cereals as bowlfuls or tablespoons
- Bread as slices (large or small loaf, thick/thin slice)
- Vegetables as tablespoons, portion size or number e.g. peas – 2 tablespoons, 1 medium carrot
- Rice/pasta as tablespoons or portion sizes (small, medium, large)

☺ If you make a dish, please give the recipe and the amount eaten.

☺ If your child takes a packed lunch to school, please record everything you put in their packed lunches and then record everything that is left after school. Also ask your child if they swapped any items or had anything else such as water which was not included in their packed lunches.

☺ If your child has a school dinner, please record what they had and ask your child what was not eaten.

The first page is given as an example on how to fill in the diary.

Meal / Snack (Time)	Food / Drink consumed	Cooking / Preparation Method	Amount consumed	Office Use Only	
Breakfast (7am)	Kellogg's Cornflakes		1 large bowlful		
	Milk – semi skimmed		On Cornflakes		
	Sugar		2 teaspoons		
Snack (10am)	Orange squash	- 1/5 squash (ASDA brand) -4/5 tap water	1 glass (250 ml)		
Lunch (1pm)	Sausages (pork and beef)	Grilled	2 medium sized		
	Mashed potato	Boiled/ mashed	1 tablespoon		
	Semi skimmed milk		In mash		
Lunch (1pm)	Mineral water	Evian	1 bottle (500 ml)		
Snack (5pm)	Tea, Twining, Assam,	- 1 teabag - Boiled tap water - Semi-skimmed milk (2 tablespoons) - Sugar, brown (1	1 mug (200 ml)		
7pm	Hot Chocolate	- 2 tablespoons chocolate powder - ½ pint semi-skimmed milk	1 mug (1/2 pint)		
8pm	Sparkling water, flavoured	- Superdrug brand	1 bottle (500 ml)		

Appendix 12 2-day duplicate instruction

The Assessment Level of Fluoride Intake/Exposure (ALFIE) using "2-day Duplicate Plate Collection" & "3-day Dietary Diary" methods

Duplicate Plate Collection Instruction Booklet

School of Health and Social Care

University of Teesside

Child ID number: ……

Record Days:

1……

2……

Instructions:

- ☺ Please keep the usual diet habits and DUPLICATE everything your child actually eats and drinks over 2 consecutive days: **one week day (Friday) and one weekend (Saturday).**

- ☺ Duplicate the diet as precisely as possible by observing the amounts that your child actually eats and drinks.

- ☺ Please remove parts of food not normally eaten, such as seeds, cores, skin and bones, before including the food in the container provided and place it in the fridge for collection.

- ☺ In the case of cooked meals, serve 2 similar portions on 2 separate plates and wait until your child finishes her/his portion. Then add food to or remove food from the comparable portions on the separate plate. Keep the plate in a container and place it in the fridge for collection.

- ☺ All drinks except milk and water can be kept in the same vial. Milk and water should be kept in different vials. All solids should also be kept together in a separate container.

- ☺ If your child receives a school dinner, there will be an arrangement with the schools for the researcher to duplicate your child's dinner.

Containers and vials will be provided for you.

We will demonstrate and show you how the food/drinks should be duplicated.

Appendix 13 Tooth brushing data record form

a	Weight of toothbrush	
b	Weight of toothbrush+ weight of toothpaste	
c	Weight of toothpaste used	(b) - (a)
	Weight of toothpaste after brushing	
	Weight of cup	
d	Weight of cup+ water(before brushing & rinsing)	
e	Weight of cup+ water (after brushing & rinsing)	
f	Weight of water	(e) - (d)
g	Weight of pot	
h	Weight of pot+ expectorated saliva	
i	Weight of expectorated saliva	(h) - (g)
	Weight of water used for TB and spatula	
	Weight of pot+ expectorated saliva + water used for rinsing TB & spatula	
j	F concentration of tap water	
k	F content of water	(j) x (f)
l	F concentration of toothpaste	
m	F content of toothpaste	(l) x (c)
n	Total F	(k) + (m)
o	F concentration of expectorated saliva	
p	F content of expectorated saliva	(o) x (i)
q	Weight of F ingested/brushing	(n) – (p)
r	Number of brushing /day	
s	Weight of F ingested/day	(q) x (r)
t	Time taken to brush teeth	
u	Toothpaste brand name	

Appendix 14 Urine instruction booklet

The Assessment Level of Fluoride Intake/Exposure (ALFIE) using "2-day Duplicate Plate Collection" & "3-day Dietary Diary" methods

Collection of a 24-hour urine sample

Information & Instruction Booklet for Parents

School of Health and Social
Care University of Teesside
Middlesbrough

Why do we collect 24-hour urine?

- Your child consumes fluoride from food and drink. The fluoride is absorbed into the body.
- The amount of fluoride retained in your child's body depends on a number of factors such as how actively they are growing.
- The fluoride which is not absorbed by your child's body will leave the body via the urine.
- Analysing the 24-hour urine helps us to find out how much of the fluoride your child took in, has been retained in the body.

Does it need to be a full 24-hour?

- It is important that we collect all the urine that your child passes in a 24-hour period.
- When the urine is collected from your child, you will be asked if it is a complete sample as this is quite important for our analysis.
- In case of incomplete urine collection you will be asked if your child is willing to repeat the urine collection.

How do you collect a 24-hour urine sample?

- The third day of the food and drink diary recording and second day of duplicate plate recording will be the day you start to collect your child's urine. You will collect urine twice as there are two methods being used for this study which require 2 urine samples.
- A **Saturday** will be the day you start to collect your child's urine.
- You are provided with a bottle containing a tiny amount (1 ml) of Chlorhexidine as a preservative (It is one of the ingredients of some of the mouth rinses available in the market and are known to kill bacteria in the mouth associated with gum disease) a funnel and a jug to collect the urine samples.
- On the morning of the 24-hour urine collection **DO NOT** collect the first sample of the urine. Your child should urinate into the toilet when he/she gets up in the morning. However, record the time of this first voided urine.
- Afterwards collect all the urine your child voids or passes each time

she/he goes to the toilet up to the time they go to bed and including the first time they go to the toilet on the Sunday morning. Collect the urine in the jug and pour it straight into the bottle with the funnel provided. Males might find it easier to collect their urine straight into the bottle in which case the jug does not need to be used. Close the bottle lid tightly and rinse the jug.

- Repeat the process each time your child goes to the toilet.
- If your child wakes up during the night and needs to go to the toilet, collect all the urine voided in the same bottle.
- On the morning of the 2nd day (Sunday), collect the first morning urine voided when your child get up and place it in the bottle. This is the end of the 24-hour urine collection period.
- Label the bottle with the date and the time of the last voided urine.

How do you store the urine?

- No special storage conditions are needed for the bottles of urine. They can be kept in a convenient place for you, such as near the toilet.
- Be sure to replace the safety cap onto the bottle tightly after each sample has been added.
- It is recommended that the bottles are kept out of the reach of the children when not in use.

When will we collect the urine samples from you? Arrangements will be made to collect the urine sample from you. This will be the day after the 24-hour collection has taken place (Sunday). When the urine collection pack is provided the exact date of urine collection will be given to you.

What will be done with the urine samples?

The urine samples will be transported to the University of Teesside where the volume of the samples will be measured. We then store a small amount (30-50 ml) of the urine in a freezer (-20ºC) at University of Teesside. The rest will be disposed of in a toilet at the University laboratory.

The samples will be analysed for their:

1. fluoride concentration at the University of Teesside by the research student;

2. potassium, nitrogen and creatinine concentration at James Cook University Hospital (Middlesbrough) laboratory by a technician.

Any Problems?

If you have any problems or concerns whilst carrying out the 24-hour urine collection, please do not hesitate to call Narges on:

University of Teesside School of Health & Social Care Tel: 01642344111
Mobile: 07925153789
E-mail: g7128303@tees.ac.uk

Appendix 15 Urine record form

Child ID	Dietary method	Urine Volume ml/d	Time of first voided urine	Time of last collection